(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 073 143 A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 82304377.3

(22) Date of filing: 19.08.82

(51) Int. Cl.³: C 07 C 103/52
C 07 D 207/16, A 61 K 31/02

(30) Priority: 21.08.81 US 295137

(43) Date of publication of application:
02.03.83 Bulletin 83/9

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(71) Applicant: UNIVERSITY OF MIAMI
Coral Gables Florida 33124(US)

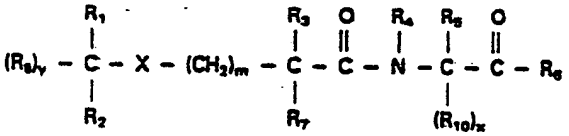
(72) Inventor: Ryan, James Walter
3420 Poinciana Avenue
Miami Florida 33133(US)

(72) Inventor: Chung, Alfred
8781 Southwest 87th. Street
Miami Florida 33183(US)

(74) Representative: De Minvielle-Devaux, Ian Benedict Peter et al,
CARPMAELS & RANSFORD 43, Bloomsbury Square
London WC1A 2RA(GB)

(54) Novel complex amido and imido derivatives of carboxyalkyl peptides and thioethers and ethers of peptides.

(57) Novel inhibitors of angiotensin converting enzyme are disclosed which have the general formula



wherein R_1 and/or R_3 form complex amides and imides thereof, X = S, O or NR_8, R_4 and R_5 form with –N–C– a 4-6 membered ring structure as described and the other R substituents are selected from a variety of disclosed groups.

EP 0 073 143 A2

Background of the Invention

Angiotensin converting enzyme (peptidyldipeptid hydrolase, hereinafter referred to as ACE) ccupi s a central r le in the physi logy of hyp rtension. Th enzym is capable of converting the decapeptide angiotensin I, having the sequence

AspArgValTyrIleHisProPheHisLeu

to an octapeptide, angiotensin II, by removal of the carboxy-terminal HisLeu. The symbols for the foregoing chemical moieties and others used throughout this application are explained in the following table:

Arg = arginine
Asp = aspartic acid
Boc = t-butyloxycarbonyl
Cbo = carbobenzyloxy
>Glu = pyro-L-glutamic acid
Gly = glycine
Hip = Hippuric acid (Benzoyl-glycine)
His = histidine
Ile = isoleucine
Leu = leucine
Phe = phenylalanine
Pro = proline
ΔPro = 3,4-dehydroproline
Ser = serine
Tos = tosyl
Trp = tryptophan
Tyr = tyrosine
Val = valine
Pht = phthaloyl
ACE = angiotensin converting enzyme
Hepes = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

In each instance the symbol for any amino acid is also used herein at times to refer to a mono-or-di-val nt radical of such acid and those of ordinary skill in the art will readily understand the context of each specific use.

Angiotensin I is formed by the action of th enzym renin, an endopeptidase found in kidney, other tissues and plasma, on a serum α-2 globulin.

Blood pressure is affected by certain peptides found in the blood. One of these, angiotensin II, is a powerful pressor (blood pressure elevating) agent. Another, bradykinin, a nonapeptide with the sequence ArgProProGlyPheSerProPheArg is a powerful depressor (blood pressure lowering) agent. In addition to a direct pressor effect, angiotensin II stimulates release of aldosterone which tends to elevate blood pressure by causing retention of extracellular salt and fluids. Angiotensin II is found in measurable amount in the blood of normal humans. However, it is found at elevat d concentrations in the blood of patients with renal hypertension.

The level of ACE activity is ordinarily in excess, in both normal and hypertensive humans, of the amount needed t maintain observed levels of angiotensin II. However, it has been found that significant blood pressure lowering is achieved in hypertensive patients by treatment with ACE inhibitors. [Gavras, I. et al., New Engl. J. Med. 291, 817 (1974)].

ACE is a peptidyldipeptide hydrolase. It catalyzes th hydrolysis of the penultimate peptide bond at the C-terminal end of a variety of acylated tripeptides and larger polypeptides having an unblocked α-carboxyl group. The acti n of ACE results in hydrolytic cleavage of the penultimate peptide bond from the carboxyl-terminal end yielding as reaction products a dipeptide and a remnant.

The reactivity of the enzyme varies markedly depending on the substrate. At least one type of peptide bond, having the nitrogen supplied by proline, is not hydrolyzed at all. The apparent Michaelis constant (Km) varies from substrat to substrate over several orders of magnitud . For general discussion of the kinetic paramet rs of enzyme catalyz d reactions, see Lehninger, A., Biochemistry, 2nd. Ed., Worth Publishers, Inc., New York, 1975, pp. 189-195. Many p p-tides which are called inhibitors of the enzymatic c nversion

of angiotensin I to angi tensin II are in fact substrat s having a low r Km than angiotensin I. Such peptides are more properly termed competitive substrates. Examples of competitive substrates include bradykinin, and the peptide BPP_{5a} (also called SQ20475) from snake venom, whose sequence is GluLysTrpAlaPro.

Numerous synthetic peptide derivatives have been shown to be ACE inhibitors by Ondetti, et al. in U.S. patent 3,832,337 issued August 27, 1974.

The role of ACE in the pathogenesis of hypertension has prompted a search for inhibitors of the enzyme that could act as antihypertensive drugs. See for example U.S. patents 3,891,616, 3,947,575, 4,052,511 and 4,053,651. A highly effective inhibitor, with high biological activity when orally administered, is D-3-mercapto-2-methylpropanoyl-L-proline, designated SQ14225, or "captopril" disclosed in U.S. patent 4,046,889 to Ondetti et al., issued September 6, 1977, and in scientific articles by Cushman, D.W. et al., Biochemistry 16, 5484 (1977), and by Ondetti, M. et al., Science 196, 441 (1977). The inhibitor SQ14225 reportedly has an I_{50} value of 2.3×10^{-8}M. The I_{50} value reported by Cushman, et al., supra is the concentration of inhibitor required to produce 50% inhibition of the enzyme under a standard assay system containing substrate at a level substantially above K_m. It will be understood that I_{50} values are directly comparable when all potential factors affecting the reaction are kept constant. These factors include the source of enzyme, its purity, the substrate used and its concentration, and the composition of the assay buffer. All I_{50} data reported herein have been performed with the same assay system and same enzyme (human urinary ACE) and with the same level of substrate and are therefore internally consistent.

The mode of action of SQ14225 has been based upon a model of the active site of ACE developed by analogy with the better known related enzym , carboxypeptidase A. The active site was postulated to have a cationic sit for binding the carboxyl end group of the substrate and a

pocket or cleft capable of binding the side chain of the C-terminal amino acid and providing specially tight binding for the heterocyclic ring of a terminal proline residue. A similar pocket for the penultimate amino acid residue was postulated, and the published data suggested a rather stringent steric requirement, since the D-form of the inhibitor was substantially more potent than its stereoisomer or the 3-methyl and unsubstituted analogs. The sulfhydryl group on the inhibitor, postulated to be bound at the active site near the catalytic center, was believed to play a central role in inactivation of the enzyme by combining with the zinc moiety known to be essential for catalytic activity. Substituents on the sulfhydryl, such as a methyl group, and a S-acetyl derivative, substantially reduced potency of the inhibitor. See Cushman, D.W., et al., Biochemistry, supra.

In vitro study of the mechanism by which SQ14225 and its analogs act to inhibit ACE has been somewhat hampered by the instability of these molecules under ambient conditions. For example, it has been observed that a fresh aqueous solution of concentration, e.g., 1 mg per ml of SQ14225 at a pH of about 8 becomes substantially less active upon standing for as little as 30 minutes, and that activity continues to decrease as the solution stands for longer periods. It is believed that this loss in activity is mainly the result of dimerization of SQ14225 occurring at the sulfhydryl end groups, whereby a disulfide is formed which is largely inactive as an inhibitor. Since the free sulfhydryl group is highly reactive and may be readily oxidized to polar acidic moieties such as sulfone and sulfoxide groups, it may also be that the observed in vitro loss of activity of aqueous solutions of SQ14225 on standing is in some part a consequence of one or more such oxidation reactions, with formation of a sulfone or sulfoxide which does not function effectively as an inhibitor for ACE.

Such reports of SQ14225 clinical testing as are currently available, some of which refer to the compound under

the name "Captopril" or "Cap ten", suggest that th product is sufficiently stable in the normal gastric and intestinal envir nments of most patients t be an eff ctive inhibitor of ACE when administered orally. It is not yet clear, however, whether there may be a group of patients for which SQ14225 is substantially ineffective. Because of the high reactivity of the free sulfhydryl group, SQ14225 could readily form mixed disulfides with serum, cellular proteins, peptides or other free sulhydryl group-containing substances in the gastric or intestinal environments, in addition to the possibility for dimer formation or oxidative degradation reactions. A mixed disulfide with protein may be antigenic and, indeed, occaional allergic reactions have been clinically observed. See Gavras, et al., New England J. Med. 298, 991 (1978). Disulfides and oxidative degradation products of SQ14225, if formed, may at best be expected to be largely ineffective as inhibitors. It may be postulated accordingly that dose response to SQ14225 may vary with conditions of administration and among individual patients. Moreoever, in at least some patients, unwanted side effects may occur and maintenance of an effective concentration of the inhibitor in the body may be difficult to control.

Adverse effects of SQ14225 in man include fevers and rashes. (Gavras et al, supra). Hoorntje et al., The Lancet, i., 1212-1214 (1980) describe the performance of renal biopsies on 13 patients treated with SQ14225. All biopsies showed evidence of immune complex deposition along the glomerular basement membranes, although 9 of 13 patients were asymptomatic at the time of the biopsy. These authors also discussed similarities of their findings with those induced by another drug with a free mercapto group, D-penicillamine.

In an effort to devise better inhibitors of angiotensin converting enzyme that are more stable than captopril and le s likely to induce D-penicillamin -like adverse effects, applicants have prepared a series of compounds having side chain structure analogous to an effective substrate for th enzyme, benzoyl-Phe-Ala-Pro and disclosed them in cop nding

U.S. application Ser. No. 187992 filed September 17, 1980. Also relevant are the class of carboxyalkyldipeptides derivatives disclosed in European published application of Patchett et al. published on or about June 25, 1980. The present application defines compounds such as N-[L-1-carboxy-3-(carbonalide)propyl]D,L-Ala-L-Pro, N-[L-1-carboxy-3-(carb-4-iodoanilide)propyl]-D, L-Ala-L-Pro, and analogs i.e., amides and imides of N-(lower alkylene)Ala-Pro. These two named compounds were found to be unexpectedly effective in inhibiting angiotensin converting enzyme in vitro, that is they have a very low I_{50}, in the order of 10^{-9}M. In contrast another closely related analog of the two named compounds, i. ., N-[L-1-carboxy-2-(carbopyrrolide)ethyl]-D,L-Ala-Pro, was found to have a much higher I_{50}, in the order of 10^{-7}M, a potency of inhibitor likely to be too low for anti-hypertensive effectiveness. It is believed, therefore, that amides and imides of N-(lower alkylene)-Ala-Pro and related compounds have unpredictable effects on angiotensin converting enzyme.

In addition, the removal of iodine from N-[L-1-carboxy-3-(carbo-4-iodoanilide)propyl]-D,L-Ala-L-Pro increases intravenous effectiveness three-fold, an unexpectedly large difference in the in vivo effect of the anti-hypertensive compounds of this invention. Hence, amides and imides of N-(lower alkylene)-D,L-Ala-Pro and related compounds are new agents with surprising effectiveness in lowering blood pressure in vivo.

Moreover, since the compounds of this invention do not have the free sulfhydryl group of SQ14225, they are most likely to be stable and have durations of action much longer than that of SQ14225. Thus, inhibitors of this invention may be used for treating hypertension with less frequent dosage schedules than required for SQ14225 and may be capable of administration under less rigorously controlled conditions.

Brief description of the invention

Novel inhibitors of ACE are disclosed which base the general formula

(vii) alkoxyphenyl or alk xybenzyl in which the alkoxy group has 1 - 3 carbons, phenoxyphenyl, phenoxybenzyl, benzyloxyb nzyl or benzyloxyphenyl or a thi ether analog of any of them;

(viii) $-(CH_2)_n-\underset{\underset{OB}{|}}{CH}-CH_3$ wherein n = 0-4 and B = H

or a 1 - 6 carbon alkyl group, or an -SB analog thereof;

(ix) $(CH_2)_pCOOZ$ or $(CH_2)_p$ COSZ wherein p = 0 - 3 and Z is H, phenyl, benzyl, a 1 - 5 carbon alkyl group, or an anion of a physiologically acceptable salt;

(x) $-(CH_2)_n-\underset{\underset{O-C-Z}{\underset{\|}{O}}}{\overset{|}{CH}}-CH_3$ or $-(CH_2)_n-\underset{\underset{C-Z}{\underset{\|}{O}}}{\overset{|}{CH}}-CH_3$

or $-(CH_2)_n-\underset{\underset{S-C-Z}{\underset{\|}{O}}}{\overset{|}{CH}}-CH_3$ or $-(CH_2)_n-\underset{\underset{C-SZ}{\underset{\|}{O}}}{\overset{|}{CH}}-CH_3$

wherein n is 0 to 4 and Z each have the same significance as above;

(xi) $HO-(CH_2)_n-\overset{D}{\overset{|}{CH}}-$ or $HS-(CH_2)_n-\overset{D}{\overset{|}{CH}}-$ wherein n = 0 - 4, D is phenyl, thienyl or a 1 - 3 carbon alkyl group;

(xii) $HO-(CH_2)_n-C(CH_3)_2-$, $HS-(CH_2)_n-C(CH_3)_2-$, p-hydroxyphenyl $-(CH_2)_n-C(CH_3)_2-$ or -p-mercaptophenyl-$(CH_2)_n-C(CH_3)_2-$ wherein n has the same significance as above;

(xiii) p -mercaptophenyl - $(CH_2)_n-CH_2-$ or p-hydroxyphenyl - $(CH_2)_n-CH_2-$ wherein the phenyl ring has one or two nitro or amino substituents and n has the same significance as above;

(xiv) $CH_3\ (CH_2)_n-\overset{OH}{\overset{|}{CH}}-$ or $CH_3\ (CH_2)_n-\overset{SH}{\overset{|}{CH}}-$ wherein n has the same significance as above;

(xv) NH_2 - alkylene r NO_2 - alkylen containing one hydroxy or mercapto substituent and having 1 - 6 carb n atoms;

(xvi) hydroxy- or mercapt -phen xyb nzyl;

(vii) $ZO(CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, $ZS-(CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$,

$NH_2-(CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, $NO_2(CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$,

$HONH - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, $NH_2NH - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$,

$ZO - \overset{O}{\overset{\|}{C}} (CH_2)_q - \overset{O}{\overset{\|}{C}} (CH_2)_n -$, $ZA - \overset{O}{\overset{\|}{C}} - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$

or $NH_2\overset{O}{\overset{\|}{C}}NH - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$ wherein q = 1 - 5 and n is from 0 to 4 and Z has the same significance as above;

(xviii) $ZO-(CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$, ,

$ZS-(CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$, $NH_2-(CH_2)_q - \overset{O}{\overset{|}{C}}H - (CH_2)_n -$,

$NO_2 - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

$NH_2 - \overset{O}{\overset{\|}{C}} - NH - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

$ZO-(CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$, $ZS - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

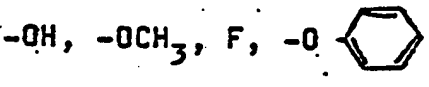
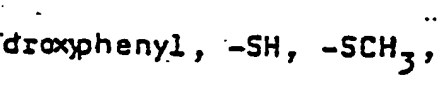
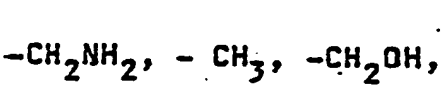
$HONH - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$, or

$NH_2NH - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

wherein q and n all have the same significance as above;

(xix) $G - NH - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, $G - NH (CH_2)_q -$

$\overset{OH}{\overset{|}{C}}H -(CH_2)_n -$, $G - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$,

it being understood that any of these structures may be monosubstituted with -OH, $-OCH_3$, F,  , Cl, Br, I, phenyl, hydroxyphenyl, -SH, $-SCH_3$,  ,  , $-NHCH_3$, $-CH_2NH_2$, $-CH_3$, $-CH_2OH$, propyl, guanidino, nitroguanidino or thioguanidino and that any of the 5- or 6-membered rings may be disubstituted with -OH, F, Cl, Br, I, OCH_3 or any combination of two of this group of substituents;

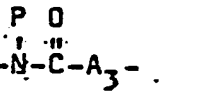
R_6 is -OM or -SM, wherein M may be H, an alkyl group of 1-3 carbon atoms or any other ester moiety hydrolyzable under mammalian <u>in vivo</u> conditions to -OH, or an ionically bonded anion of a physiologically acceptable nontoxic salt;

R_7 is H-, CH_3-, halomethyl, hydroxymethyl, aminomethyl or mercaptomethyl;

R_8 is H-, CH_3-, amino, halomethyl, hydroxymethyl, aminomethyl, dihalomethyl, trihalomethyl, mercaptomethyl, methoxymethyl, methylthiomethyl, methoxycarbonylmethyl, cyanomethyl, benzyl, acetoxymethyl, $CH_2=CH-CH_2$-, isobutyl, mercaptoalkyl of 2-3 carbon atoms, hydroxyalkyl of 2-3 carbon atoms, acetylthioethyl, benzamido, acetamido, phthaloylaminoalkylene wherein the alkylene group has 1-4 carbon atoms, α-alkoxycarbonyl isoalkylene wherein the alkyl group contains 1-5 carbons the isoalkylene group contains 3 - 5 carbons, benzoylamine, alkanoylamine of 1 - 5 carbons, alkylamide of 1 - 5 carbons, phenylamine, alkylamine of 1 - 5 carbons, or ethyl;

and

A. R_1 and R_3 may each be of the general formula



wherein A_3 is:

(i) alkylene of 1-6 carbons, branched chain alkyl of 1-6 carbons, cycloalkyl alkylene, alkylcycloalkylalkylene, or alkylcycloalkylene;

(ii) aralkylene wher in th alkyl group is 1-6 carb ns or alkylaryl;

(iii) phenyl;

(iv) alkylaralkylene wherein th alkyl groups may be the same or different and are 1-6 carbons in length;

(v) substituted alkylene, substituted branched chain alkyl, substituted cycloalkylalkylene, substituted alkyl cycloakkylalkylene, substituted alkylcycloalkylene, substituted alkylaryl, substituted aralkylene, substitut d phenyl or substituted alkylaralkylene wherein the substituent or substituents may be the same or different, may be included in an alkylene chain or pendent thereto, and are selected from amino, halo, hydroxy, mercapto, NO_2, carboxy, $CONH_2$, lower alkyl, halomethyl, hydroxymethyl, aminomethyl, dihalomethyl, trihalomethyl, cyano, mercaptomethyl, methoxymethyl, methylthiomethyl, methoxycarbonylmethyl, cyanomethyl, benzyl, acetoxymethyl, $CH_2=CH-CH_2-$, isobutyl, mercaptoalkyl of 2-3 carbon atoms, hydroxyalkyl of 2-3 carbon atoms, acetylthioethyl, benzamido, acetamido, phthaloylaminoalkylene wherein the alkylene group has 1-4 carbon atoms, -alkoxycarbonyl isoalkylene wherein the alkyl group contains 1-5 carbons and the isoalkylene group contains 3-5 c. rbons, benzoylamino, alkanoylamino of 1-5 carbons, alkylamide of 1-5 carbons, phenylamine, alkylamine of 1-5 carbons, low r alkoxy, aryloxy, lower alkylamino, diloweralkylamino, acylamino, arylamino, guanidino, imidazolyl, indolyl, lower alkylthio, arylthio, carboxy amido and carbolower alkoxy;

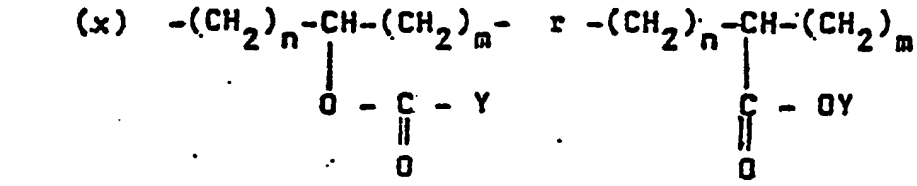
(vi) alkylenethio- or alkylenethioalkylene of 1-6 carbons, alkylthioalkylene of 1-6 carbons;

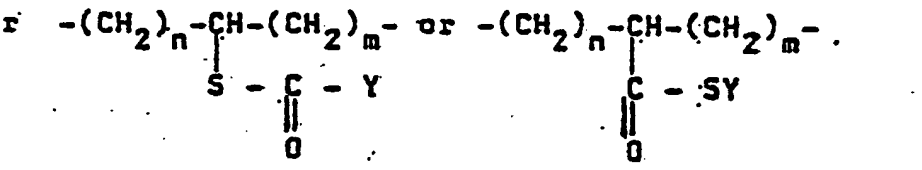
(vii) alkyleneoxy or alkyleneoxyalkylene wherein the alkyl groups may be the same or different and are 1-6 carbons;

(viii) alkoxyphenyl or alkoxybenzyl in which the alkoxy group has 1-3 carbons, phenoxyphenyl, phenoxybenzyl, benzyloxybenzyl or benzyloxyphenyl or a thioether analog of any f them;

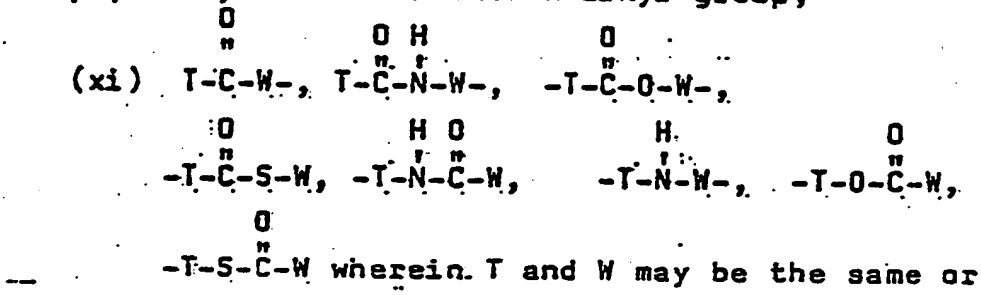
(ix) $-(CH_2)_n-\underset{B}{CH}-(CH_2)_m-$ wherein n=0-4, m=0-4 and B=H

or a 1-5 carbon alkyl group; or an -SB analog th reof;

(x) 

or 

wherein n and m have the same significance as above, Y is phenyl, benzyl or a 1-5 carbon alkyl group;

(xi) 

-T-S-C(=O)-W wherein T and W may be the same or different and are alkylene, aryl, benzyl or cycloalkyl; and P and Q may be the same, or one of them may be H or they may combine to form a ring with the nitrogen to which they ar attached.

Either or both of P and Q may be selected from any of the following:

(a) C_1-C_6 straight or branched chain alkyl groups or C_1-C_6 straight or branched chain alkenyl groups, any one of which may be substituted with any of halo, hydroxy, alkoxy, aryloxy, amino, alkylamino, dialkylamino, alkylacylamino, arylamino, guanidino, thioguanidino, nitroguanidino, hydrazino, ureido, nitro, mercaptocarbonyl, hydroxyamino, histidinyl, cyano, imidazolyl, indolyl, mercapto, alkylthio, arylthio, carboxy amido or carboalkoxy, wherein the alkyl groups contain 1-6 carbon atoms;

(b) cycloalkyl or cycloalkyl alkylene wherein cycloalkyl has 4-12 carbons, and alkylene 1-5 carbons, which may be substituted with any of -OH, -SH, halo, COOH, COSH, $CONH_2$,

NO_2NH_2, NO_2, CH_3, $-OCH_3$, $-SCH_3$, $-\overset{O}{\overset{\|}{C}}-OCH_3$, hydrazino, ureido, hydroxyamino, cyano, guanidin , thioguanidino or nitroguanidin groups;

(c) aralkyl or alkaryl groups which may be ring substituted with one or more of the following:

SH, halo, CH_2COOH, CH_2CONH_2, CH_2CONH-alkyl, COSH, COOH, $CONH_2$, CONH-alkyl, CH_2COSH, CH_2SH, CH_2OH, OH, NO_2, amin , alkyl, alkoxy, aralkyloxy, alkylthio and aralkylthio gr ups, wherein the alkyl groups contain 1-6 carbons and may als or alternatively be chain substituted with $-CH_3$, $-OH$, $-OCH_3$, halo, $-SCH_3$, $-\overset{O}{\overset{\|}{C}}-CH_3$, $-NH_2$, $-NO_2$, $-CN$, $-SH$, $-NHOH$, $-NHNH_2$, $NH-\overset{O}{\overset{\|}{C}}-NH_2$ or a thio or nitro derivative thereof, -COOH or COSH;

(d) an aryl, heterocyclic or adamantanyl group which may be ring-substituted with at least one group selected from halo, $-OH$, $-O$-alkyl, $-O$-aryl, NH_2, NH-alkyl, $N-(alkyl)_2$, $alkyl-\overset{O}{\overset{\|}{C}}-NH_2$, $aryl-NH_2$, guanidino, thioguanidino, nitro-guanidino, hydrazino, ureido, nitro, mercaptocarbonyl, hydroxyamino, cyano imidazolyl, indanyl, histidinyl, $-SH$, $-S$-alkyl, S-aryl, $-\overset{O}{\overset{\|}{C}}-NH_2$, $-\overset{O}{\overset{\|}{C}}-O$-alkyl, $-\overset{O}{\overset{\|}{C}}-$alkyl, $-\overset{O}{\overset{\|}{C}}-O$-aryl, $-\overset{O}{\overset{\|}{C}}-$aryl, $-\overset{O}{\overset{\|}{C}}-SH$, $-\overset{O}{\overset{\|}{C}}-S$-alkyl, $-C-S$-aryl and $-NO_2$

when P and Q join with N to form a ring, the ring may b any 4-10 membered heterocyclic ring which contains a nitrogen with only two of its valences attached to other ring members.

B. Alternatively R_1 may be

$Q-\overset{P}{N}-\overset{O}{\overset{\|}{C}}-A_3-$ and

R_3 may be

(i) mono-N sub tituted alkylene of 2-4 carbons wh rein the N substituent is benzoyl, Boc, CbO, Tos, formyl or acetyl;

(ii) hydroxphenyl or hydroxyphenyl-(1-6C)-alkylene or a thiol analog of ither,

(iii) mercapt alkylene of 1-6 carbons;

(iv) phenylalkylene wherein the alkylene group has 1-6 carbons;

(v) phenylthioalkylene or benzylthioalkylene wherein the alkylene group has 1-6 carbons;

(vi) alkylthioalkylene wherein the alkyl and alkylene groups have 1-3 carbons;

(vii) alkoxyphenyl or alkoxybenzyl in which the alkoxy group has 1-3 carbons, phenoxyphenyl, phenoxybenzyl, benzyloxybenzyl or benzyloxyphenyl or a thioether analog of any of them;

(viii) $-(CH_2)_n-\underset{\underset{OB}{|}}{CH}-CH_3$ wherein n=0=4 and B=H or a 1-6 carbon alkyl group; or an -SB analog thereof;

(ix) $(CH_2)_p COOZ$ or $(CH_2)_p COSZ$ wherein p = 0 - 3 and Z is H, phenyl, benzyl, a 1 - 5 carbon alkyl group, or an anion of a physiologically acceptable salt;

(x) $-(CH_2)_n - \underset{\underset{O-\underset{\underset{O}{\|}}{C}-Z}{|}}{CH} - CH_3$ or $-(CH_2)_n - \underset{\underset{\underset{\underset{O}{\|}}{C}-Z}{|}}{CH} - CH_3$

or $-(CH_2)_n - \underset{\underset{S-\underset{\underset{O}{\|}}{C}-Z}{|}}{CH} - CH_3$ or $-(CH_2)_n - \underset{\underset{\underset{\underset{O}{\|}}{C}-SZ}{|}}{CH} - CH_3$

wherein n is 0 to 4 and Z each have the same significance as above;

(xi) $HO-(CH_2)_n - \underset{\underset{D}{|}}{CH}-$ or $HS-(CH_2)_n - \underset{\underset{D}{|}}{CH}-$ wherein n = 0 - 4, D is phenyl, thienyl or a 1 - 3 carbon alkyl group;

(xii) $HO-(CH_2)_n - C(CH_3)_2 -$, $HS-(CH_2)_n - C(CH_3)_2 -$, p-hydroxyphenyl $-(CH_2)_n - C(CH_3)_2 -$ or -p-mercaptophenyl-$(CH_2)_n - C(CH_3)_2 -$ wherein n has the same significance as above;

(xiii) p-mercaptophenyl - $(CH_2)_n$ - CH_2 - or p-hydroxyphenyl - $(CH_2)_n$ - CH_2 - wherein the phenyl ring has one or two nitro or amino substituents and n has the same significance as above;

(xiv) $CH_3(CH_2)_n - \overset{OH}{\overset{|}{C}}H -$ or $CH_3(CH_2)_n - \overset{SH}{\overset{|}{C}}H -$ wherein n has the same significance as above;

(xv) NH_2 - alkylene or NO_2 - alkylene containing one hydroxy or mercapto substituent and having 1 - 6 carbon atoms;

(xvi) hydroxy- or mercapto-phenoxybenzyl;

(xvii) $ZO(CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n - ZS(CH_2)_q - O - (CH_2)_n -$,

$NH - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, $NO_2(CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$,

$HONH - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, $NH_2NH - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$,

$ZO - \overset{O}{\overset{\|}{C}}(CH_2)_q - \overset{O}{\overset{\|}{C}}(CH_2)_n -$, $ZS - \overset{O}{\overset{\|}{C}} - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$

or $NH_2\overset{O}{\overset{\|}{C}}N - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n$ - wherein q = 1 - 5 and n is from 0 to 4 and Z has the same significance as above;

(xviii) $ZO(CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$, $ZS(CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

$NH_2 - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$, $NO_2 - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

$NH_2 - \overset{O}{\overset{\|}{C}} - NH - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

$ZO(CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$, $ZS - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

$HONH - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$, or $NH_2NH - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

wherein q and n all have the same significance as above;

(xix) $G - NH - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, $G - NH(CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

$G - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, $G - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

$NH_2 - \overset{O}{\overset{\|}{C}} - (CH_2)_q - \overset{O}{\overset{\|}{C}} - (CH_2)_n -$, or $NH_2 - \overset{O}{\overset{\|}{C}} - (CH_2)_q - \overset{OH}{\overset{|}{C}}H - (CH_2)_n -$,

wherein G is an alkacyl or alkacyloxy group of 1 - 6 carbons, a benzoyl or benzyloxy group, or a phenylalkacyl or phenylalkayloxy group wherein the alkacyl or alkacyloxy group contains 2 - 6 carbons and q and n have the same significance as set forth above;

(xx) $K-(CH_2)_n-\overset{O}{\overset{\|}{C}}-(CH_2)_n-$ or $K(CH_2)_n-\overset{OH}{\overset{|}{CH}}-(CH_2)_n-$

wherein n has the significance stated above and K is selected from carboxyphenyl, aminophenyl, nitrophenyl, halphenyl, hydroxyphenyl, alkylthiophenyl, alkylphenyl, mercaptophenyl, cyanophemyl, mercapto-carbonylphenyl, alkylcarbonylphenyl, alkylcarbonyloxyphenyl, hydriazinophenyl, ureidephenyl, alkylcarbonylaminophenyl, alkylcarbonylthiophenyl, alkyloxyphenyl and hydroxy-aminophenyl, wherein all alkyl groups contain 1 - 6 carbon atoms;

(xxi) $L-(CH_2)_n-\overset{O}{\overset{\|}{C}}-(CH_2)_n$ or $L(CH_2)_n-\overset{OH}{\overset{|}{CH}}-(CH_2)_n-$

wherein n has the significance stated above and L is selected from cycloalkyl groups of 3 - 7 carbons which may be unsubstituted or substituted with up to two groups selected from among carboxy, amino, nitro, halo, hydroxy, mercapto, mercaptocarbonyl, hydroxyamino, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkylthio, alkylcarbonylamino, alkylcarbonylthio, cyanohydrazino, ureido and alkyloxy, wherein all alkyl groups contain 1 - 6 carbon atoms;

(xxii) guanidino alkylene, thioguanidinoalkylene or nitroguanidino alkylene in which the alkylene groups contain 1 - 6 carbon atoms;

(xxiii) ring substituted aryl groups in which the ring substituents may be the same or different and may comprise up to five per ring of the following: - NH_2, -OZ, -SZ, halogen, -CN, -NO_2, -COOZ, -COSZ, $CONH_2$, - $NHNH_2$, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkylcarbonylamino, haloalkyl, dihaloalkyl, trihalomethyl, hydroxyamino, alkylcarbonylthio, phenoxy, and benzyloxy wherein the alkyl groups contain 1 - 6 carbon atoms and Z has the same significance as above;

(xxiv) amidoalkylene or alkylcarbonyl-aminoalkylene wh rein the alkyl and alkylene groups contain 1 - 6 carbon atoms;

(xxv) hydroxyaminoalkylen of 1 - 6 carb ns;

(xxvi) vinyl and substituted vinyl groups in which th substituents may be alkyl, aryl, cycloalkyl or heterocyclic groups;

(xxvii) unsubstituted heterocyclic groups from among phenothiazinyl, pyrrolidinyl, pyrrolyl, quinolinyl, imidazolyl, pyridyl, thyminyl, benzothiazinyl, indolyl, thienyl, purinyl, piperidinyl, morpholinyl, azaindolyl, pyrazinyl, pyrimidyl, piperonyl, piperazinyl, furanyl, thiazolyl and thiazolidinyl, cytosinyl;

(xxviii) alkylene or alkenyl groups of 1 - 6 carbons substituted with one of the heterocyclic rings from (xxvii) above;

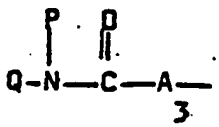
(xxix) groups from (xxvii) or (xxviii) above containing up to four ring substituents on the heterocyclic ring selected from among - OZ, - SZ, - COOZ, - NO_2, $-NH_2$, - COSZ, halogen, haloalkyl, dihaloalkyl, trihalomethyl, cyano, $CONH_2$, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkylcarbonylamino, alkyl-carbonylthio, phenoxy, benzyloxy, $-NH-\overset{\overset{O}{\|}}{C}-NH_2$, $-NHNH_2$ and HONH -, wherein Z has the same significance as above;

(xxx) groups from (xxvii), (xxviii) or (xxix) attached to one valence of an etheric -O- or -S-;

(xxxi) mono-, di- or tri-alkyl, alkenyl - or phenyl-silyl or -selenyl wherein the alkyl or alkenyl groups contain 1 - 6 carbons;

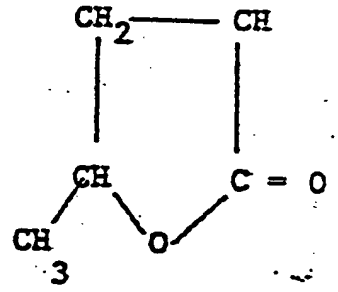
(xxxii) any of H, 1 - 5 carbons straight or branched chain alkyl, phenyl, -OH, alkoxy of 1 - 6 carbons, benzyloxy, benzyloxyalkylene or phenoxyalkylene wherein the alkylene has 1 - 5 carbons, alkoxyalkylene having 1 - 5 carbons in the alkoxy and alkylene groups, aminoalkylene of 1 - 6 carbons, alkenyl of 1 - 6 carbons, b nzyl, hydroxyalkyl of 1 - 6 carbons, mercaptoalkyl of 1 - 6 carbons, histidinyl, haloalkyl of 1 - 6 carbons, 4 - aminom thyl-benzyl, acetamidoalkyl of 1 - 5 carbons, benzylthiomethylene, or dimethylaminoalkyl of 1 - 5 carbons.

C. Alternatively, R_3 may be

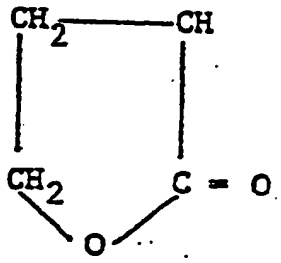


and

R_1 may be any of groups (i) - (xxxi) above or any of H, C_1 - C_8 straight or branched chain alkyl, phenyl, benzyl, unsubstituted aminoalkylene of 2 - 6 carbons, hydroxyalkylene of 1 - 6 carbons, hydroxyphenyl, phenoxyalkylene or benzyloxyalkylene wherein the alkylene group has 1 - 6 carbons; cycloalkyl of 3 - 6 carbons, cycloalkyl methyl, 3 indolyl-, phenylethyl, methylthioethyl 3 indolyl alkyl wherein the alkyl group contains 1 - 5 carbons, imidazolyl, imidazolylalkyl wherein the alkyl group contains 1 - 5 carbons, phenoxymethyl, phenylthiomethyl, 4-aminomethyl benzyl, 2-amin - phenethyl, naphthylethyl, 4-halophenethyl, 3,4-dihalophenethyl or phenoxyphenethyl, or R_1 and R_2 together may form with -CH a lactone ring of the formula:



or



or an analogous six-membered ring.

In the general formula above, asterisks indicats possible asymmetric centers. These centers may be racemized or in any optically active form. However, the S-form is preferred.

The inhibitors are useful as orally effective anti-hypertensive agents.

Detailed description of the invention

The present invention in its broad aspects relates to thioether, ether and secondary amino compounds containing at least one amino acid or related structure containing the

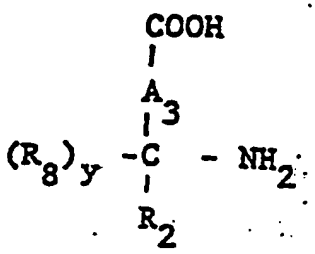
sequence N-C(=O)-C-R_6, preferably N-CH-C(=O)-R_6, and include at least one group of the general formula Q-N(-P)-C(=O)-A_3- in the R_1 or R_3 position.

The compounds of this invention wherein X = NR_9 may be made in a variety of ways. For example, an alpha keto carboxylic acid of the general formula R_3-C(=O)-COOH may be coupled to $HN(R_4)-C(R_5)(-(R_{10})_x)-C(=O)-R_6$ to give a product $R_3-C(=O)-C(=O)-N(R_4)-C(R_5)(-(R_{10})_x)-C(=O)-R_6$ using a conventional coupling agent such as dicyclohexylcarboiimide ("DCC") or diphenylphosphorylazide ("DPPA"). This product in turn may be coupled, in the presence of a reducing agent such as sodium cyanoborohydride to a compound of the general formula $(R_8)_y-C(R_1)(R_2)-NHR_9$ to give the desired compound.

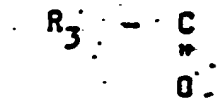
In such an instance Q-N(H)-P may, e.g. be first reacted with an appropriate ω-carboxylated compound, e.g.,

$(R_8)_y-C(A_3)(R_2)-NH_2$ to yield $A_3-C(=O)-N(P)-Q$ $(R_8)_y-C(R_2)-NH_2$

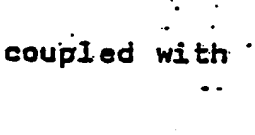
In this particular scheme, e.g.,



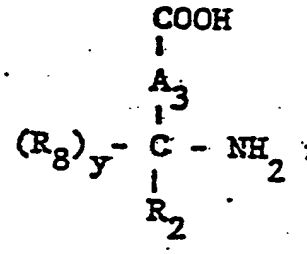
may alternatively first be coupled with



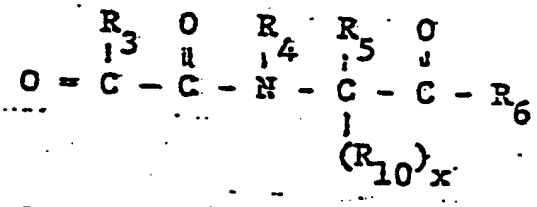
and the product then coupled with



in such instances Q - N - P may be reacted with the -COOH attached to A_3 after the first or second coupling step. Similarly,



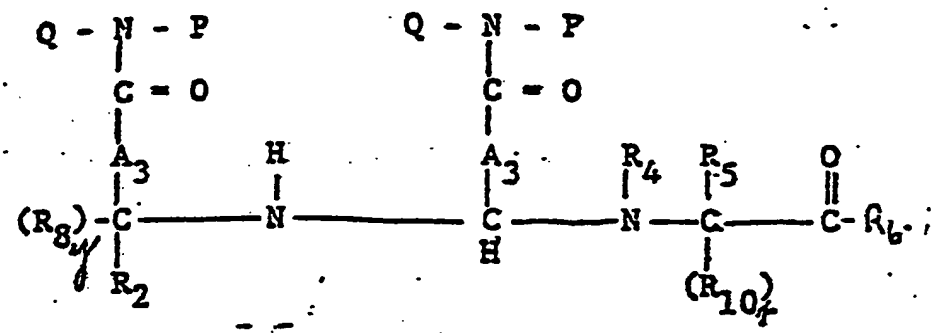
may be coupled to

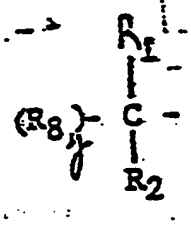


and Q - N - P may then be reacted with the COOH adjacent to A_3. As those of ordinary skill in the art will readily understand, conventional blocking groups such as BOC, CbO, etc. may be introduced at appropriate stages to protect reactive groups and may be removed when protection is no longer needed or wanted.

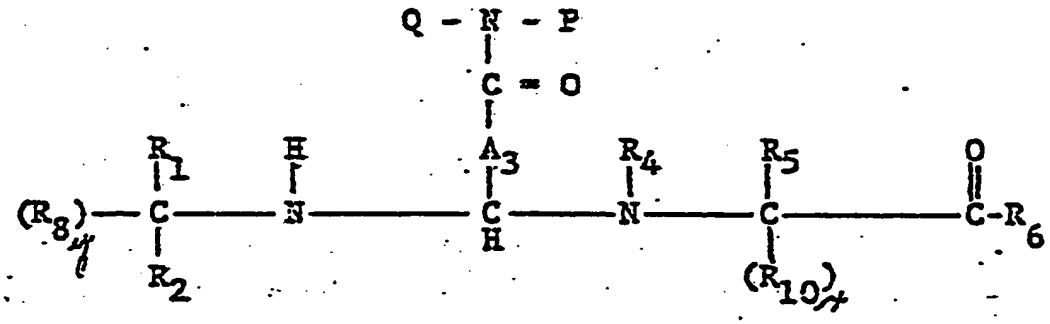
It is within ordinary skill, e.g., to use in lieu of

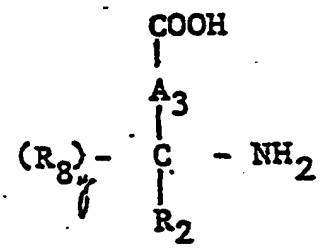
a compound HOOC - A_3 - - COOH, protect either of the COOH groups as desired in th particular reaction scheme preferred and prepare,



Similarly if  wherein

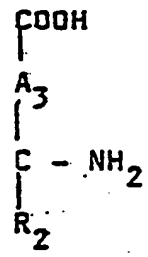
R_1 is other than $A_3-\overset{O}{\overset{\|}{C}}-N(P)-Q$ is chosen and $HOOC-A_3-\overset{O}{\overset{\|}{C}}-COOH$ is used in lieu of $R_3-\overset{O}{\overset{\|}{C}}-COOH$, the reactions may be manipulated with appropriate blocking and coupling steps t yield a product



Among suitable ω-carboxylic acids of the general formula  are

glutamic acid α-benzyl ster
glutami acid α- thyl ester
glutamic acid α-methyl est r
glutamic acid α- t - butyl ester
aspartic -α - benzyl ester
2-amino - 5-carboxy - indan -2-carboxylic acid
para-carboxy phenyl alanine α-methyl ester
ortho-carboxy tyrosine α- methyl-ester
2-amino malinic acid monoethyl ester
2-amino adipic acid 1-ethyl ester
2-amino pimelic acid 1-ethyl ester
2-amino suberic acid 1-ethyl ester
2-amino azelaic acid 1-ethyl ester
2-amino sebacic acid 1-ethyl ester and others which will readily occur to those of ordinary skill in the art.

These acids may be purchased in many instances from, e.g. Aldrich Chemical Co. or Chemical Dynamics Co. It is also well known that α-amino acids of the formula



wherein R_2 is

COOH or another carboxyl function may be obtained from α-keto dicarboxylic acids using methods described by Waters, K.L., Chem. Rev. 41, 585-98 (1947).

Among suitable compounds of the general formula $HOOC-A_3-\overset{\overset{O}{\|}}{C}-COOH$ referred to above are:

α-keto glutaric acid
oxalacetic acid
ket malonic acid
4-keto pimelic acid
para-carboxy phenyl pyruvic acid
indole-1-carboxy-3-pyruvic acid
β-carboxy-DL-lactic acid
2-ketoadipic acid

It is to be understood that when $R_3 = Q\text{-}\overset{P}{N}\text{-}\overset{O}{\overset{\|}{C}}\text{-}A_3$, the compound of the general formula $(R_8)\text{-}\overset{R_1}{\overset{|}{C}}\text{-}NHR_9$ can be selected from a very wide group.

Suitable R_1 compounds of the general formula $(R_8)\text{-}\overset{R_1}{\overset{|}{C}}\text{-}$ $\underset{R_2}{|}$ NHR_9 for use in making the compounds of the invention include, but are not limited to tert.leucine, 2-methylglutamic acid, α-amino-γ-guanidino butyric acid, α-amino-β-guanidino-propionic acid, β-fluorophenylalanine, β-hydroxyvaline, α-oxa-lysine, 3-hydroxy ornithine, N^{ϵ}-hydroxylysine, -N^{ω}methyl arginine, N^{γ}-hydroxyarginine, canvanin, 5,5'-dihydroxyleucine, β-carboxyaspartic acid, β-fluoroaspartic acid, β-methyl-aspartic acid, β-methylene aspartic acid, p-amido phenyl-alanine, p-guanidinophenylalanine, p-methyl-phenylalanine, 2-ethoxy-5-nitrophenyl-alanine, 2-hydroxy-5-nitrophenyl-alanine, 4-mercaptophenylalanine, 2-amino-2-indoleacetic acid, 2-amino-3-adamantylpropionic acid, β-methylene norvaline, α-amino- -(4 carboxythiazolyl)-butyric acid) 3-chloroglutamic acid, α-amino-γ-nitrovaleric acid, 4-azalysine, β-(2,4,5-trihydroxyphenyl)-alanine, β(3-bromo-5-methoxyphenyl) alanin , β- (3,5 dimethyl - 4 methoxyphenyl) alanine, 3,5-di (ethylthio)-4-(4' hydroxphenoxy) - phenylalanine, 3,5-di (ethylthio) -4(3' -isopropyl-4'-methoxyphenoxy)-phenylalanine, β-pyrrolyl-alanine, 2-amino-4-pyrrolyl-butyric acid, 2-amino -5-pyrrolyl-valeric acid, β -(2 pyridyl) alanine, β-(3 pyridyl) alanine, β(6-aminopurin - 9yl) alanine, β-(4-amino-2-hydroxy-pyrimidin -1-yl) alanine, β-(2,4 dihydroxy - 5 methyl - pyrimidin-1-yl) alanine, β-(6-hydroxy-purin -9-yl) alanine, β(6-dimethylamino-purin -9 yl) alanine, β-(6-mercaptopurin-9 yl) alanine, β-(6-methylthiopurin -9yl) alanine, 4-azatrypto-phan, 4-methyl- 6-chloro-7-azatryptophan, N^{ϵ}-(1,4-dehydro -6 methyl -3-hydroxy -4-oxo- 1 pyridyl) lysine, S-(2-hydr xy -2-carboxyethanethiomethyl) - cystein , 2-amino -3- (6-thieno

[3,2-b] pyrrolyl) propionic acid, 3,3',5,5' t trimethyl thyronine, 3-hydroxy -L-lysin , 2-aminoh x-4-ynoic acid, N-hydroxyornithin , 4-piperazinobut - 2-ynoic acid, 4-pip ridinobut -2-yn ic acid, 4-pyrrolidinobut -2-ynoic acid, α-amino -N^{γ}-nitroguanidinobutyric acid, α-amino β(1-imidazolyl) propionic acid, 4-nitrohistidine, 2-methyl -3 (2', 4'-diiodo 5' -hydroxyphenyl) alanine, 4-(3'amino-2', 4', 6'-triiodophenyl) - isovaline, 4-(3' acetamido -2', 4', 6' - triiodophenyl) - isovaline, 4-(3'-hydroxy -2'; 4', 6'-triiodophenyl) isovaline, 2-amino-4-thiosulfobutyric acid, S-(3-aminopropyl) homecysteine, S-(cyclopentyl methyl) homocysteine, 5' -guanosyl homocysteine, β (cytosin-1-yl)- alanine,

S-[(diphenyl-α-naphthyl)methyl]-L-cysteine,

S-[(diphenyl-β-naphthyl)methyl]-L-cysteine,

2-amino-6-(methylthio)caproic acid,

$N^{G}N^{G}$-dimethyl-L-arginine,

$N^{G}N'^{G}$-dimethyl-L-arginine,

$N^{\varepsilon}N^{\varepsilon}N^{\varepsilon}$-trimethyl-δ-hydroxy-L-lysine,

N^{ε}-(5-amino-5-carboxypentyl)-5- hydroxy-L-lysine,

δε -dihydroxy-L-norleucine,

cis-1-amino-1,3-dicarboxycyclohexane,

trans-1-amino-1,3-dicarboxycyclohexane,

3,3,4,4,4,-pentafluoro-2-aminobutyric acid,

3,3,4,4,5,5,5,-heptafluoro-2-aminovaleric acid,

ω-fluoro-DL-and L-allo-isoleucine,

2,6-diamino-4-hexynoic acid,

O-(α-D-glucopyranoxyl)-L-serine,

2-amino-5,6-dihydroxyindan-2-carboxylic acid,

3-(m-fluorophenyl)-2-methylalanine,

3-(m-bromophenyl)-2-methylalanine,

3-(m-iodophenyl)-2-methylalanine,

2-[(m-iodophenyl)methyl]glycine,

4-(m-iodophenyl)-2-methyl-2-aminobutyric acid

3,5,3'-tri-isopropyl-DL-thyronine,

3,5-dimethyl-3'-is propyl-thyronine

3,5-di-isopropyl-thyronine,

3,5-di-isopropyl-4'-amino-thyronine,
3,5-di-isopropyl-3'-bromo-thyronin ,
3,5-di-isopropyl-3'-methyl-thyronine,
3,5-di-s-butyl-thyr nine,
3,5-di-s-butyl-4'-amino-thyronine,
3,5-di-s-butyl-3'-bromo-thyronine,
3,5-di-s-butyl-3'-iodo-thyronine,
4-fluoro-tryptophan,
5-fluoro-tryptophan,
6-fluoro-tryptophan,
β-5(-hydroxy-6-iodo-2-pyridyl)-alanine,
β-(benzimidazol-5-yl)-alanine,
β-(2-amino-6-hydroxypurin-9-yl)-alanine,
β-(2-amino-6-mercaptopurin-9-yl)-alanine,
N^{ε}-(5-Amino-6-chloro-4-pyrimidyl)lysine,
α-Amino-ε-(6-chloro-9-purinyl)caproic acid,
4-Fluoro-DL-histidine,
S-Methyl-2-methyl-cysteine,
S-Ethyl-2-methyl-cysteine
S-propyl-2-methyl-cysteine,
S-Isopropyl-2-methyl-cysteine,
S-Butyl-2-methyl-cysteine,
S-Isobutyl-2-methyl-cysteine,
S-t-Butyl-2-methyl-cysteine,
S-Amyl-2-methyl-cysteine,
S-Isoamyl-2-methyl-cysteine,
S-Allyl-2-methyl-cysteine,
S-(β-Aminoethyl)homocysteine,
γ,δ,δ'-trihydroxy-leucine,
N^{ε}-(indole-3-acetyl)-lysine,
p-hydroxymethylphenylalanine,
O-ethylhomoserine,
5-methyl-2-aminohex-4-enoic acid,
α-(3-hydroxyphenyl)glycine,
α-(3,5-dihydroxyphenyl)glycine,
β-(cycl hexa-1,4-dienyl)alanine,
β-(cyclohex-1-enyl)-alanin ,
β-(1-hydroxycyclohexyl)-alanin ,
4-bromoacetyl-phenylalanine,

4-bromoacetamido-phenylalanine,
3-chloracetamido-phenylalanine,
4-fluor-3-chloroacetamido-phenylalanine,
3,4,5-tri-iodo-phenylalanine,
3,5-di-isopropyl-3'-iodo-thyronine,
β-(4-methoxy-1-naphthyl)-α-methylalanine,
β-(4-hydroxy-1-naphthyl)-α-methylalanine,
α-(2-indanyl)glycine,
β-trimethylsilyl-alanine,
α-amino-β-(methylamino)propionic acid,
$N^{\varepsilon}N^{\varepsilon}$-bis(2-cyanoethyl)-lysine,
α,γ-dimethylnorleucine,
α-methyl-$N^{\varepsilon}N^{\varepsilon}$-diethylornithine,
α-ethyl-3,4-dimethoxy-phenylalanine,
α-methyl-4-morpholino-phenylalanine,

β-(2-amino-4-pyrimidinyl)alanine,
3-(2-Methyl-4,5-dihydroxyphenyl)-alanine,
3-(2-Ethyl-4,5-dihydroxyphenyl)-alanine,
3-(2-Isopropyl-4,5-dihydroxyphenyl)-alanine,
3-(2-t-Butyl-4,5-dihydroxyphenyl)-alanine,
3-(2,5-Dimethoxy-4-methylphenyl)-alanine,
3-Ethyl-α-methyl-tyrosine,
2-amino-3,3-dimethylhex-5-enoic acid
2-aminohexa-4,5-dienoic acid
2-amino-3,3-dimethylhexa-4,5-dienoic acid,
2-aminohepta-4-5-dienoic acid,
2-amino-3,3-dimethylhepta-4,5-dienoic acid,
2-amino-3,3-dimethylnona-4,5-dienoic acid,
2-aminohepta-5,6-dienoic acid,
2-amino-3-methylhepta-5,6-dienoic acid,
2-amino-5-t-butyl-6,6-dimethylhepta-3,4-dienoic acid
2-amino-5-methylhepta-3,4-dienoic acid
2-aminohept-4-en-6-ynoic acid
ε-hydroxy-β-carboxy-norleucine,
β-carboxy-lysine,
β-(3,4-dihydroxyphenyl)-α-methyl-serine
S-benzyl-β,γ-dimethyl-homocysteine,

S-benzyl-α-γ,γ-trim thyl-hom cysteine,
β-methyl-methionine,
α-methyl-selenomethionine,
β-methyl-L-sel nomethi nine,
γ-methyl-selenomethionine,
γ,γ'-difluoro-valine,
δ,δ'-difluoro-leucine,
γ-fluoro-allothreonine,
β-hydroxy-asparagine,
β-hydroxy-isoleucine,
β-methoxy-isoleucine,
αamino-γ-(methylamino)butyric acid,
α-amino-β-(ethylamino)propionic acid,
3-Isopropyl-α-methyl-tyrosine,
3-t-Butyl-α-methyl-tyrosine,
2-Amino-5-hydroxy-indan-2-carboxylic acid,
2-Amino-5-methoxy-indan-2-carboxylic acid,
2-Amino-5-carboxy-indan-2-carboxylic acid,
2-Amino-5-chloro-indan-2-carboxylic acid,
2-Amino-5-bromo-indan-2-carboxylic acid,
2-Amino-5-iodo-indan-2-carboxylic acid,
3-(2,4-Difluorophenyl)-alanine,
3-(3,4-Difluorophenyl)-alanine,
3-(3,5-Difluorophenyl)-alanine,
3-(2,5-Difluorophenyl)-alanine,
3-(2,6-Difluorophenyl)-alanine,
3-(2,3,5,6-Tetrafluorophenyl)-alanine,
3-(3,5-Dichloro-2,4,6-trifluorophenyl)-alanine,
3-(2,3,4,5,6-Pentafluorophenyl)-alanine,
β-(1,2-Dihydro-2-oxo-3-pyridyl)-alanine,
β-(1,2-Dihydro-2-oxo-4-pyridyl)-alanine,
β-(1,2-Dihydro-2-oxo-5-pyridyl)-alanine,
β-(1,2-Dihydro-2-oxo-6-pyridyl)-alanine,
β-(2-Fluoro-3-pyridyl)-alanine,
β-(2-Fluoro-5-pyridyl)-alanine,
β-(2-Fluor -6-pyridyl)-alanine,
β-(2-Bromo-3-pyridyl)-alanine,
β-(2-Bromo-4-pyridyl)-alanine,

β-(2-Br mo-5-pyridyl)-alanine,
β-(2-Br mo-6-pyridyl)-alanin ,
β-(2-Chl ro-3-pyrydyl)-alanine,
β-(2-Chloro-4-pyridyl)-alanine,
β-(2-Chloro-5-pyridyl)-alanine
β-(2-Chloro-6-pyridyl)-alanine,
β-(Thymin-1-yl)-alanine,
It is further contemplated that

$(R_8)_y - \underset{\underset{R_2}{|}}{\overset{\overset{R_1}{|}}{C}} - NHR_9$ may be

selected from among any of the known amino acids or esters or from amides thereof in which, when R_1 is any of CH_3, $NH_2-(CH_2)_3$, $(NH_2(CH_2)_4-$, $CH_3S(CH_2)_2-$, benzyl-, p-hydroxy-benzyl, 3,4-dimethoxybenzyl, $CH_3\overset{O}{\overset{\|}{O}}C-(CH_2)_2-$, or

R_8 is selected from among

CH_3-, $(CH_3)_2CH-CH_2-$, Pht $N(CH_2)_3-$, $CH_2 = CH - CH_2-$, benzyl-, nitrilomethylene-, ethyl $CH_3O-\overset{O}{\overset{\|}{C}}-CH_2-$,

CH_3OCH_2-, CH_3SCH_2-, $-CH_2F$, $-CHF_2$, $-CF_3$,

$-CH_2Cl$, $-CF_2Br$, PhtN $(CH_2)_2-$, $CH_3\overset{O}{\overset{\|}{C}} - S(CH_2)_3-$,

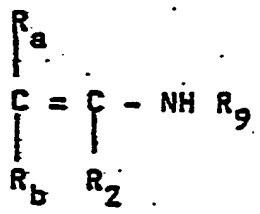
$HS(CH_2)_3-$, or CH_3CH-

$C = O$

OC_2H_5

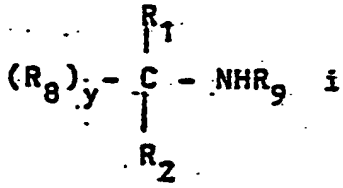
There are also known aminoacids, and esters or primary amides there f in which, wh n R_8 is hydroxymethyl, R_1 may be methyl, ethyl, isopr pyl, isobutyl, phenyl, b nzyl r methylthio thyl.

It is als contemplated that r actants of th g n ral formula



wherein

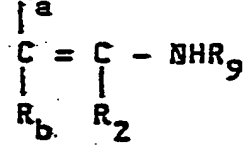
R_2 is COOH may be utilized in lieu of



in the coupling reaction with

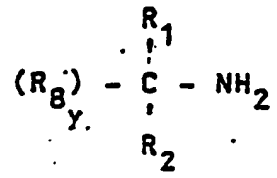
or its coupling product already described.

In such case,



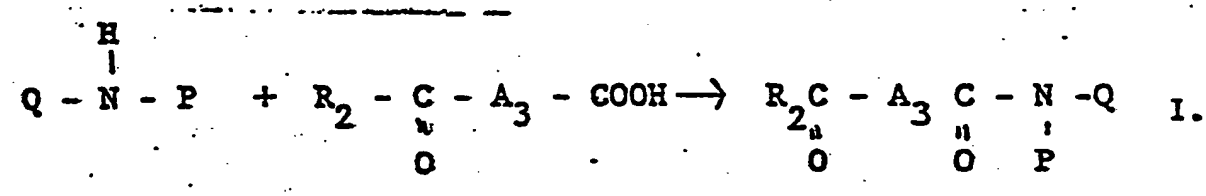
may be, e.g., dehydroalanine, α,β-dehydrophenylalanine, vinyl glycine or a known compound in which R_a and R_b are both methyl or ethyl or R_a is phenyl or a substituted phenyl group such as 3,4-dimethoxyphenyl and R_b is methyl. In this instance various functional groups such as halo, hydroxy or mercapto groups and their methylene analogs, may later be added to one or both carbons of the unsaturated bond via well known and conventional organic chemical procedures.

Many suitabl variations in



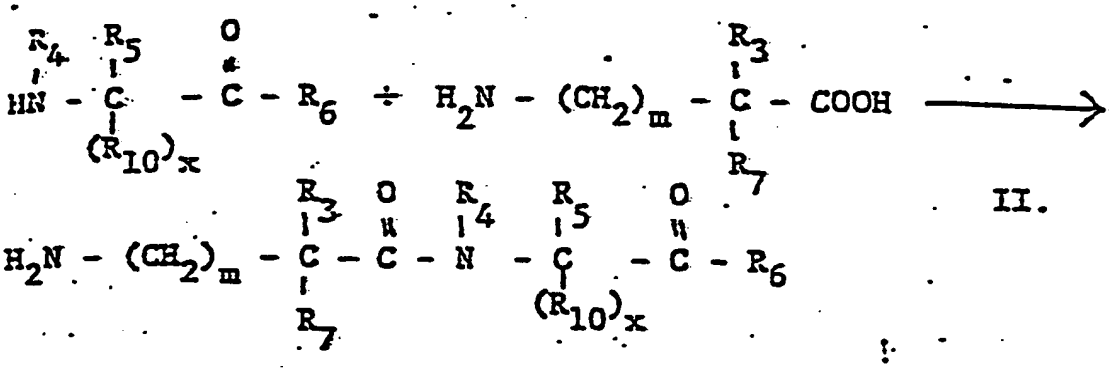
will readily occur to those of ordinary skill in the art.

Another general method for synthesizing compounds of this invention is to couple a suitable α keto carboxylic acid with a suitable dipeptide derivative. A suitable α keto acid can be formed in the reaction,



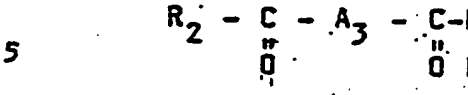
I.

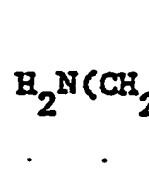
in the presence of a conventional coupling agent. An appropriate dipeptide derivative can be formed in the reacti n

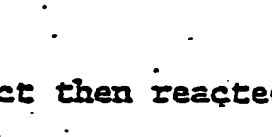


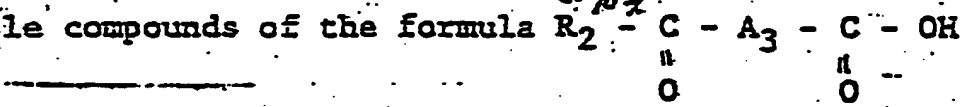
II.

Compounds of this invention are then obtained by reacting I and II. Alternative schemes are readily apparent, for example,

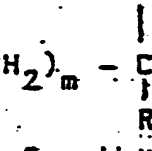
 may be reacted with



and the product then reacted with  .

Suitable compounds of the formula 

for use in this synthesis method include, but are in no sense limited to, acetoacetic acid, 5-aminolevulinic acid, acetobutyric acid, acetyl cyclopentanecarboxylic acid, chloromethylketocyclopentane carboxylic acid, dibromomethylketocyclohexanecarboxylic acid, 1-acetyl-4-piperidinecarboxylic acid, N-acetyltryptophan, p-carboxyphenoxyacetic acid, 2-benoylbenzoic acid, 4-benzoylbenzoic acid, 4-benzoylbutyric acid, 3-benzoylpropionic acid, mercaptoacetophenone-4-carboxylic acid, hydroxyethylbenzoyl benzoic acid, the pyruvoyl alkyl carboxylic acids, and others which will readily occur to those of ordinary skill in the art.

Suitable compounds of the formula,  include, but are by no means limited to 2-methalalanine, histidine, N -acetyl-lysine, tryptophan, α-methyltryptophan, albizziin, 2-amino-adipic acid, p-aminophenylalanine, phenylalanine, arginine, aspartic acid, asparagine, 2-methylglutamic acid, N -hydroxylysine, 2-amino-3-adamantyl propionic acid, α-hydroxymethylalanine, α-methyl methionine, α-Methyl-N,N-diethylornithine, α-methyl-4 morpholinoph nylalanine, β-(4 methoxy-1-naphthoyl) α-methylalanin , and ß-(4-hydroxy-1 naphth yl)α-m thylalanine, α-ethyl-3,4-dimethoxy phenylalanine andothers which will readily occur to those of ordinary skill in the art.

So long as R_1 is of the formula $Q-\overset{P}{\underset{R_1}{N}}-A_3-$, any compound of the general formula $(R_8)_y-\overset{}{\underset{R_2}{C}}-NHR_9$ given above, wherein $R_9 = H$, and $R_2 = COOH$ may be used as $H_2N(CH_2)_m-\overset{R_3}{\underset{R_7}{C}}-COOH$.

In these cases R_1 becomes R_3, R_8 becomes R_7 and $m = 0$.

Useful compounds of the type $Q-\overset{H}{N}-P$ include amino-acenaphthene, para-morpholinoaniline, piperidine, phenyl-piperidine, hydantoin, alloxazine, rhodanine, morpholine, aminophenanthrene, adenosine, adamantanamine, adenine, C-aminoacridine, C-aminopyrimidine, aminoanthracene, amino-anthraquinone, aminoantipyrine, aminophenol, aminonaphthalene, aminobenzophenone, C-aminobenzothiadiazole, C-aminobenzo-thiazole, benzothiazole, aminobiphenyl, C-aminopyridine, C-aminothiazole, pyrazole, C-aminopyrazole, C-aminobenzox-azole, C-aminopurine, aminochrysene, aminocyclopentane, aminocyclopropane, aminocyclobutane, aminocyclohexane, aminocycloheptane, aminocyclooctane, aminocyclononane, aminocyclodecane, C-amino-benzimidazole, C-aminopteridine, N-aminopiperidine, C-amino-1,2,4-triazine, C-aminouracil, uracil, C-amino,N,N-dimethyluracil, aminodiphenylmethane, N-aminoethylimidazoline, N-aminoethylmorpholine, C-amino-morpholine, N-aminoethylpiperazine, C-aminopiperazine, N-aminoethylpiperidine, 3-amino-N-ethylpiperidine, 2-amino-ethylpyridine, N(aminoethyl)-pyrrolidine, pyrrolidine, aminofluoranthene, 1-, 2-, or 4-aminofluorenone, aminohexane, aminopentane, N-aminohomopiperidine, homopiperidine, 1-amino, 4-(βhydroxyethyl) piperazin , amino-9-hydroxyfluorene, 2-amino-4-hydroxy-6-methylpyrimidine, 4-amino-6-hydroxy-pyrazole, 4-aminoimidaz le, aminoindan, C-aminoindazole, C-aminoindole, 1- or 5- aminoisoquinoline, 3-amino-m rcapto-1,

2, 4-triazole, 4-aminobutanol-1, 5-amin pentanol-1, 2-amino-methyl-1- thylpyrrolidine, 5-aminoisothiazole, 2-amino-6-methylm rcaptopurine, 6-aminoh xanol-1, 1-amin -4-methyl-piperazine, 4-aminomethylpip ridine, 2-amin -1, 3, 4-thia-diazole, 2-amino-4-methyl thiazole, N-aminomorpholine, 2-amino-4-morpholine-s-triazine, 4-amino-1,8-naphthalimid , 6-aminonicotinamide, 5-amino-6-nitroquinoline, 2-amino-5-nitrothiazole, 6-aminopenicillanic acid, 4-aminophenyl ether, 2(p-aminophenyl)-6-methylbenzothiazole, 3-amino-1-phenyl-2pyrazoline-5-one, 3-aminophthalhydrazide, N-amino-phthalimide, 2-aminopecoline, N-aminopiperidine, 3-amino-propanol-1, N-(3aminopropyl) morpholine, N-(3 aminopropyl), ethanolamine, N(3-aminopropyl) pyrrolidinone, 2-amino-6-purinethiol, aminopyrazine, 3-aminopyrazole, 4-aminopyrazolo-pyrimidine, aminopyrene, 4-aminoquinaldine, N-aminorhodanin , 4- or 5-aminosalicylic acid, 5-aminotetrazole, tetrazole, 2-aminothiazoline, aminovaleric acid, aniline, 3,4-dimeth xy-aniline, aminoxylene, benzisooxazole, o- or p- aminobenzamid , o- or p- aminobenzoic acid, o- or p-aminobenzonitrile, 8-aza-6-aminopurine, 2-azacyclooctanone, 3-azabicyclononane, 2-azacytidine, 5-azacytosine, cytosine, 6-azacytosine, 5- or 6-azauracid, Azetidine, aminoazulene, barbituric acid, aminobenzofluorene, C-aminobenzofuran, benzothiazinone, benzylpiperazine, bis(2-ethoxyethyl)amine, bromoguanine, bromoisatin, ε-caprolactam, carbazole, tryptophan, glycine, glycinamide, glycinanilide, oxazolidine, oxazolidinone, 8-chlorotheophylline, chlorzoxazone, creatinine, aminocycl - heptadiene, aminocyclooctatriene, aminocyclooctratetraene, cycloserine, cytidine, cytosinecarboxylic acid, dehydeo-abietylamine, 4,5-diaminoacenaphthene, aminobenzidine, aminothiophene, dimethylhydantoin, aminofuran, N,N-diethyl-ethylenediamine, aminotoluene, aminoindenone, ethyl-4-amino-5-imidazole carboxylate, α-methyltryptamine, glutamine, glutathione, glutarimide, guanine, guanosine, histamine, dodecamethyleneimine, h mocarnosine, dithiouracil, 2,2'-dipyridylamine, 2,5—dimethyl-3-pyrroline, 2,6-dim thyl-piperazine, isoamarine, glycouril, leucinol, leucenol, myrtanylamine, nicotinamide, homopiperazin , isonicotinamide,

6-β-hydroxy thylamino pureine, amin n rbornan , amin norbornene, or tic acid, oxindole, phenoxazine, proline, phthalimide, pyrimidone, pyrol , <Glu, thiazolidine, triacanthine, and 1,2,4-triazole. The various compounds named can be substituted with, e.g., -OH, halo, dihalomethyl, trihalomethyl, -SH, O-alkyl, S-alkyl, phenyl, O-phenyl, S-phenyl, COOY, alkylcarbonyloxy, ureido, cyano, hydroxylamino, alkyl, alkoxyalkyl, alkoxyphenyl, phenoxyphenyl and the like. These compounds are illustrative, rather than limiting, as to suitable Q - N - P compounds.

It will be understood that $H_2N(CH_2)_m - \overset{A_3}{\underset{R_7}{C}}(COOH) - COOH$ may be utilized in this particular scheme and the A_3COOH converted to $A_3 - \overset{O}{C} - N(P) - Q$ at any desired stage of the synthesis process.

-- A variety of known methods can be employed to esterify or block any carboxyl group of a multi-carboxyl amino acid or an α-keto carboxylic acid. See, for example, Schroder E. et al, The Peptides Vol 1, Academic Press (1965) pp. 181-207, and Merrifield, R.B., Adv. Enzym. 32, 221 (1969). Furthermore, many of these precursors can be obtained commercially, e.g., from Chemical Dynamics, South Plainfield, N.J., or from Bachem Chemical Co., Torrance, California.

Another method for synthesizing compounds of Formula I involves the use of a diazomethyl intermediate. See, for example, Boyer, J.H. et al Chem. Rev. 54, 1-57 (1954); Aldrichimica Acta, 3(4) 9 (1970) an article available from Aldrich Chemical Co., Milwaukee, Wisconsin; Lieber, E. et al, Chem. Rev. 65,377-384 (1965); L'Abbe, G. Chem. Rev. 69. 345-363 (1969). This method is especially useful for synthesizing comp unds of the invention wherein $A_3 = -CH_2-$. Typically a carboxylic acid is reacted with diazomethane via a mixed anhydride reaction, e.g.,

$$R - \overset{O}{\overset{\|}{C}} - OH + CH_2N_2 \longrightarrow R - \overset{O}{\overset{\|}{C}} - CH_2 - N_2$$

the product is then reacted with an acid such as HBr or HCl, in a solvent such as ethyl acetate, to form an α-haloket n as follows:

$$R - \overset{O}{\overset{\|}{C}} - CH_2N_2 \xrightarrow{HBr} R - \overset{O}{\overset{\|}{C}} - CH_2 - Br$$

The α-haloketone can then be reacted with an equival nt of diethylformamidomalonate, then decarboxylated in aque us HCl to form derivatives of 2-amino-4-keto carboxylic acid, that is compounds of the formula

$$R - \overset{O}{\overset{\|}{C}} - CH_2 - \overset{H}{\overset{|}{C}} - COOH.$$
$$\qquad\qquad\qquad\qquad\qquad\quad NH_2$$

Compounds of this general formula can then be coupled with compounds of general formula

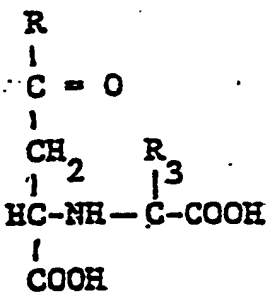
$$R_3 - \overset{O}{\overset{\|}{C}} - \overset{R_4}{\overset{|}{N}} - \overset{R_5}{\overset{|}{C}} - \overset{O}{\overset{\|}{C}} - R_6$$
$$\qquad\qquad\qquad\qquad\quad (R_{10})_x$$

in the presence of a reducing agent such as sodium cyanob ro-hydride in aqueous solution with an organic solvent (for example CH_2Cl_2 or $CHCl_3$) to form compounds of the invention.

Alternatively, $R - \overset{O}{\overset{\|}{C}} - CH_2 - \overset{H}{\overset{|}{C}} - COOH$
$\qquad\qquad\qquad\qquad\qquad\qquad\quad NH_2$

35

can be coupled with $R_3-\overset{O}{\overset{\|}{C}}-COOH$ to form



which in turn is coupled with $HN-\underset{(R_{10})_x}{\overset{R_4\ R_5}{\overset{|\ \ |}{C}}}-\overset{O}{\overset{\|}{C}}-R_6$ in the presence of DCC or DPPA to form a compound of this invention.

The diazomethyl intermediate can be formed with virtually any carboxylated organic compound. Thus, $R-\overset{O}{\overset{\|}{C}}-OH$ can be a difunctional or trifunctional amino acid, any dicarboxylic acid or any carboxylic acid. Appropriate protecting groups may also be necessary.

The thioether compounds of this invention can be produced by several methods of synthesis. In the examples of synthesis which follow for the thioether as well as the ether and secondary amine compounds, proline will be utilized as prototype amino acid moiety. It is to be understood that this is done for illustration purposes only and that the other ring structures $\underset{(R_{10})_x}{\overset{R_4\ R_5}{\overset{|\ \ |}{N-C}}}$ can be substituted for proline in these methods unless noted otherwise. According to one preferred method the compound $R_1\overset{R_8}{\overset{|}{C}}(OH)COOC_2H_5$ is reacted with P_2S_5 to form the compound $R_1\overset{R_8}{\overset{|}{C}}(SH)COOC_2H_5$.

The compound $CH_2=C(R_3)COCl$ is reacted with Pro to form

$$CH_2=C(R_3)-\overset{R_7}{\underset{}{}}-\overset{O}{\overset{\|}{C}}-Pro.$$

The two products of these reactions are reacted together to yield the compounds

$$R_1\overset{R_8}{\underset{}{C}}(COOC_2H_5)S-CH_2-\overset{R_7}{\underset{}{C}}(R_3)-\overset{O}{\overset{\|}{C}}-Pro.$$

Saponification removes the ethyl alcohol radical and forms the corresponding salt. The free acid can be formed therefrom by acidification. In this method, Δ Pro cannot be substituted for Pro.

In a second preferred method, the compound

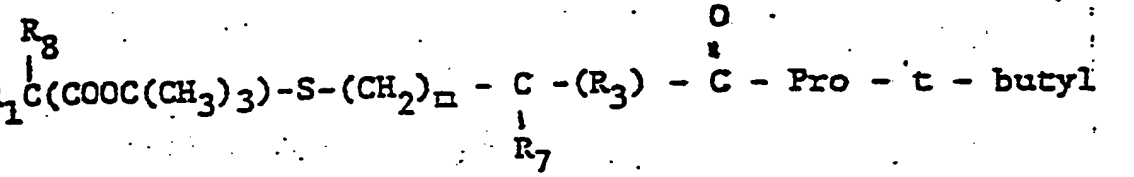
$$R_1\overset{R_8}{\underset{}{C}}(Br)COOH$$

is reacted with isobutylene in the presence of sulfuric acid to form the t-butyl ester of $R_1\overset{R_8}{\underset{}{C}}(Br)COOH$. This compound is then reacted with $R_3\overset{R_7}{\underset{}{C}}(COOH)(CH_2)_m-SH$ to form the product:

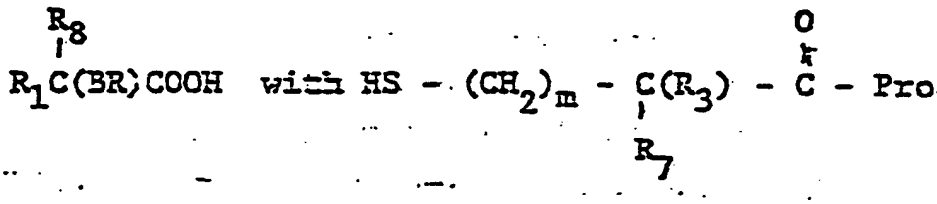
$$R_1\overset{R_8}{\underset{}{C}}(COOC(CH_3)_3)-S-(CH_2)_m-\overset{R_7}{\underset{}{C}}(R_3)-\overset{O}{\overset{\|}{C}}OH.$$

Next, the t-butyl ester of Pro is coupled to this product using conventional coupling methods, such as the dicyclohexylcarbodiimide (DCC) method. Other useful coupling methods include the mixed anhydride, symmetrical anhydride, acid chloride, active ester, Woodward reagent K, or the like, methods. For a review of the coupling methods, see Methoden der Organischen Chemie (Houben-Weyl), Vol. XV, part II, page 1 et seq. (1974).

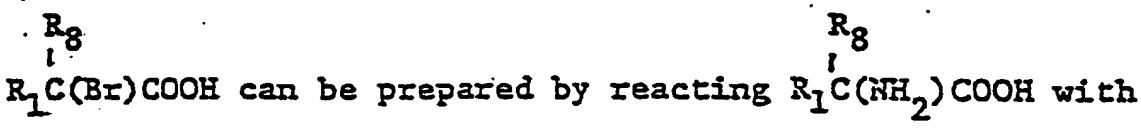
The product formed,



ester, is deprotected, i.e., the t-butyl ester groups are removed by conventional means such as treatment with trifluoroacetic acid (TFA) and anisole to produce the desired product. For a review of other deprotecting methods, see Methoden der Organischen Chemie (Houben-Weyl), Vol. XV, part I, page 376 et seq. (1974). An alternative method is the reaction of



to form the desired product. This alternative method will not work for Δ Pro although the original method will.

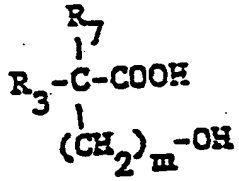


HBr in the presence of $NaNO_2$ or with KB_r in 2.5 N H_2SO_4 in the presence of $NaNO_2$ to yield the desired product.

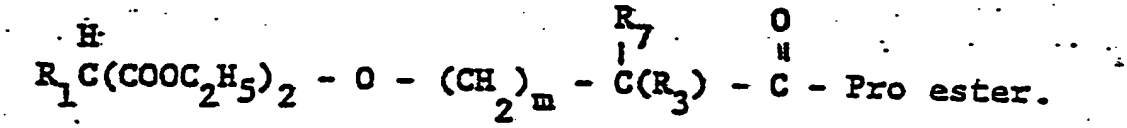
The ether compounds of formula I can also be prepared by several other methods. According to one preferred method, the compound R_1CH_2Cl is reacted with the diethyl ester of

malonic acid to yield $R_1-C(COOC_2H_5)_2$. This product is then reacted with bromine to produce $R_1-C(Br)(COOC_2H_5)_2$.

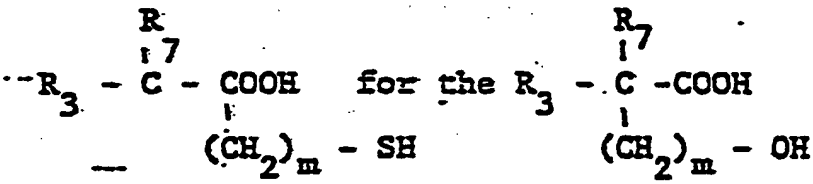
Th compound



is coupled to an ester of Pro using conventional coupling means. These two products are then reacted to form the compound

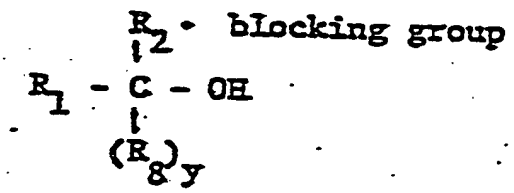


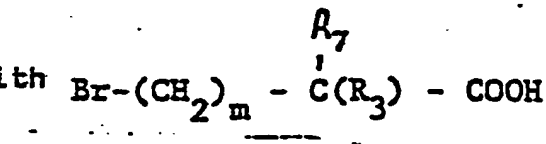
The various ester groups are removed by conventional means and one carboxyl group is removed by acidification and heat produce the desired product. By substituting

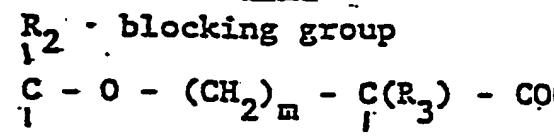


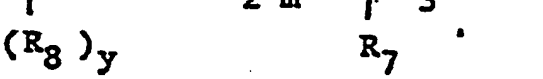
and following this procedure, the thioether compounds can be formed but this will not work with Δ Pro.

In still another method, the compound



is reacted with  t-butyl ester to



produce  t-butyl ester

Th ester i removed, th product reacted with Pro, and the blocking group is removed to yield a final pr duct according to thi inv nti n.

The secondary amine compounds of this invention wherein R_8 is H and X is NH can also be synthesized by the following method. The compound $R_1C(=O)-COOH$ is coupled with thiophenol using the mixed anhydride method to produce

$$R_1-C(=O)-C(=O)-S-C_6H_5.$$

This product is then reacted with

$$H_2N(CH_2)_m - C(R_7)(R_3) - C(=O) - Pro,$$

to yield

$$R_1C(H)(C(=O) - S - C_6H_5) - NH - (CH_2)_m - C(R_7)(R_3) - C(=O) - Pro.$$

This compound is reacted with NaSH to form

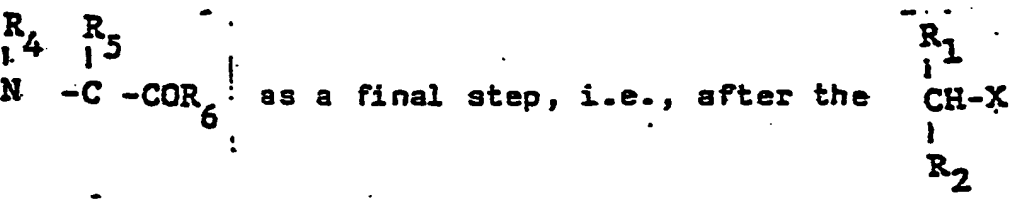
$$R_1 - CH(C(=O) - SH) - NH - (CH_2)_m - C(R_7)(R_3) - C(=O) - Pro.$$

Compounds of this invention in which R_1 and R_2 are bridged to form a lactone ring can be prepared using 2-halo-lactones, e.g., α -Br- γ -valerolactone and α -Br- γ -butyrolactone. The α-bromo group is reactive with

$$HS-(CH_2)_m-CH(R_3)-C(=O)-N(R_4)-CH(R_5)-COR_6$$

or those analogs in which an NH_2- or OH-group is substituted for the HS-group to form compounds in which X is -S, -O- or -NH- in the general formula for compounds of this invention.

A Pro cannot be used in this procedure unless added as



as a final step, i.e., after the

bond has been formed. The lactone ring can be opened, e.g., with a base such as $Ba(OH)_2$ to form the corresponding γ-OH-1-carboxymethyl compounds. The hydroxy-group can be converted to a salt with sodium, potassium or an organic cation such as that from arginine, or can be converted to an ethyl or methyl ester.

Compounds R_1 -C(=O)-COOH or R_3 -C(=O)-COOH used in any of the procedures disclosed herein may be selected from known ketocarboxylic acids, including, but not limited to,

pyruvic acid,
phenylpyruvic acid,
3-cyclohexyl-2-oxopropionic acid,
6-methyl-2-oxoheptanoic acid,
4-methyl-2-oxopentanoic acid,
2-oxobutyric acid,
3-methyl-2-oxobutyric acid,
2-oxoglutaric acid,
2-oxoadipic acid,
2-oxo-4-phenylbutyric acid,
4-(3-indolyl)-2-oxobutyric acid,
N-acetylaminoethyl-2-oxo-4-phenylbutyrate,
dimethylaminoethyl-2-oxo-4-phenylbutyrate,
2-oxo-5-methylhexanate,
phenoxypyruvic acid,
phenylthiopyruvic acid,
4-p-chlorophenyl-2-oxobutyrate,
indole-3-pyruvic acid,
2-oxo-3-p-cyan phenylpropionate,
4-α-naphthyl-2-oxobutyrate,
4-(3,4-dichlorophenyl)-2-oxo-butyrat), or
2-oxo-4-p-phenoxyph nylbutyric acid.

The compounds of this invention have one or more asymmetric carbons as indicated by the asterisks in the general formula. The compounds accordingly exist in stereoisomeric forms or in racemic mixtures thereof. All of these are within the scope of the invention. The above described syntheses can utilize a racemate or one of the enantiomers as starting material. When the racemic starting material is used in the synthetic procedure or a racemic mixture results from the synthesis, the stereoisomers obtained in the product can be separated by conventional chromatographic or fractional crystallization methods. In general the S- isomer with respect to the carbon bearing R_1 constitutes the preferred isomeric form. Also the S-isomer of the carbon bearing R_5 is preferred.

The compounds of this invention form basic salts with various inorganic and organic bases which are also within the scope of the invention. Such salts include ammonium salts, alkali metal salts like sodium and potassium salts (which are preferred), alkaline earth metal salts like the calcium and magnesium salts, salts with organic basis, e.g., dicyclohexylamine, benzathine, N-methyl-D-glucamine, procaine salts, salts with amino acids like arginine, lysine, and the like. The non-toxic, physiologically acceptable salts are preferred.

The salts are formed in conventional manner by reacting the free acid form of the product with one or more equivalents of the appropriate base providing the desired cation in a solvent or medium in which the salt is insoluble, or in water and removing the water by freeze drying. By neutralizing the salt with an insoluble acid like a cation exchange resin in the hydrogen form (e.g., polystyrene sulfonic acid resin like Dowex 50) or with aqueous acid and extraction with an organic solvent, e.g., ethyl acetate, dichloromethane or the like, the free acid form can be obtained, and, if desired, another salt formed.

Additional experimental details are found in the examples which are preferred embodiments and also serve a models for the preparation f other m mbers of the group.

The compounds f this invention inhibit the conversi n of the decapeptide angiotensin I to angiotensin II and therefore are useful in reducing or relieving angiotensin related hypertension. The action of the enzyme renin on angiotensinogen, a pseudoglobulin in blood plasma, produces angiot nsin I. Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II. The latter is an active pressor substance which has been implicated as the causative agent in various forms of hypertension in various mammalian species, e.g., rats and dogs. The compounds of this invention intervene in the angiotensin $\xrightarrow{\text{(renin)}}$ angiotensin I $\xrightarrow{\text{(ACE)}}$ angiotensin II sequence by inhibiting angiotensin converting enzyme and reducing or eliminating the formation of the pressor substance angiotensin II. Thus the administration of a composition containing one or a combination of compounds of formula I including their physiologically acceptable salts, angiotensin-dependent hypertension in the species of mammal suffering therefrom is alleviated. A single dose, or in some cases up to two to four divided daily doses, provided on a basis of about 0.03 to 20 mg per kilogram per day, is appropriate to reduce blood pressure. The substance is preferably administered orally, but parenteral routes such as subcutaneous, intra-muscular, intravenous or intraperitoneal can also be employed.

The compounds of this invention can be utilized to achieve the reduction of blood pressure by formulating them in compositions such as tablets, capsules or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration. About 10 to 500 mg of a compound or mixture of compounds of formula I, including the phy iologically acceptable salts th reof, is compounded with a physiologically acceptable vehicle, carrier, excipient, binder pres rvative, stabilizer, flav r, etc., in a unit dosage form as called for by accepted pharmaceutical practice.

The amount of active substance in these compositions or preparations is such that a suitable dosage in the range indicated is obtained.

Illustrative of the adjuvants which may be incorporated in tablets, capsules and the like are the following: a binder such as gum tragacanth, gum acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, lactose or saccharin; a flavoring agent such as peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil.

Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange flavor. Anti-oxidants may also be added. Suitable antioxidants are α-tocopherol nicotinate, vitamin A, C, E and analogs of vitamin E known in the art, retinal palmitate and other antioxidants known in the art as food additives such as the gallates.

Sterile compositions for injection can be formulated according to conventional pharmaceutical practice by dissolving or suspending the active substance in a vehicle such as water, a naturally occurring vegetable oil like sesame oil, coconut oil, peanut oil, cottonseed oil, etc., or a synthetic fatty vehicle like ethyl oleate or the like. Buffers, preservatives, and the like can be incorporated as required.

The present invention will be further described by the following examples. All temperature are in degrees Celsius unless otherwise indicated. Molar equivalents of the reactants as usually utilized.

Example 1

Synthesis of 3-N-[1-carboxy-2-(para-ethylaminocarb nyl ph nyl)ethyl]-aminopropanoyl-L-5-ketoproline

A.. 20 mmoles of 3-N-(benzyloxycarbonyl)-aminopropanoic acid is dissolved in CH_2Cl_2 at 0°C. 20 mmoles of N-hydroxysuccinimide is added and then 20 mmoles of DCC is added dropwise to this mixture. The reaction mixture is stirred for 30 minutes at 0°C and then overnight at 4°C. Crystallin dicyclohexylurea is removed by filtration. The solvent from the filtrate is removed under reduced pressure. The resulting product is dissolved in cold THF and then the solution is added to a cold solution of 20 mmoles of L-glutamic acid and 40 mmoles of $NaHCO_3$ in THF/water. The reaction mixture is stirred overnight at room temperature and then the THF is removed with a rotary evaporator. The glutamyl residue is cyclized to give the L-5-keto-proline residue according to Gibian H. and Klieger E., Justus Liebig's Ann.Chemie 640, -145 (1961). The benzyloxycarbonyl group is removed by treatment with hydrogenolysis.

B. A solution of 10 mmoles of this product and 50 mmoles of 2-keto-3-(4-ethylaminocarbonyl-phenyl) propionic tert butyl ester in ethanol is stirred with powdered molecular siev at room temperature for 1/2 hour. A solution of 40 mmol s of sodium cyanoborohydride in ethanol is slowly added over the course of six hours. The reaction mixture is filter d. The t-butyl ester is removed by treatment with trifluoroacetic acid in anisole. The named product is obtained aft r removal of the solvent with a rotary evaporator.

Example 2

Synthesis of N-[L-1- carboxyl (2-proplyaminocarbonyl-ethyl)-1-carboxyethyl]-D,L-Ala-L-Pro

A. 200 mmoles of propylamine and 150 mmoles of the α-ethyl ester of N -Boc aspartic acid are dissolved in 600 ml of cold dimethyl formamide and 125 mmoles f DPPA.. A volum of 25 ml of triethylamine in DMF is added drop-wise, holding th temperatur at about -10°C for two hours. The reaction is stored overnight at room temp rature and rotary

evaporated t remove DMF. The product is 3-(propylamino-carbonyl)-2-aminopropanoic acid thyl e t r. Th B c group i removed with TFA.

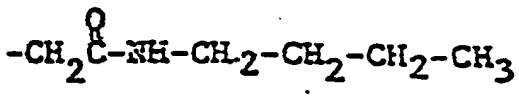
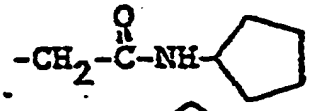
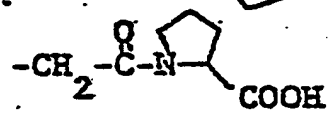
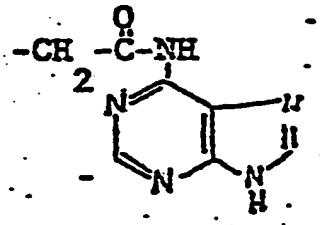
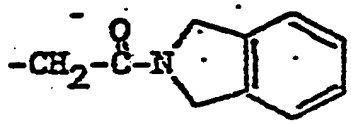
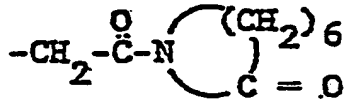
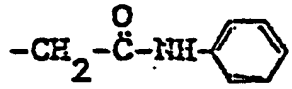
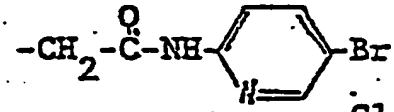
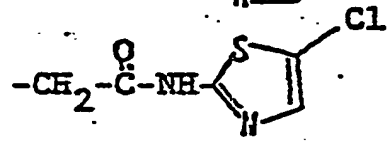
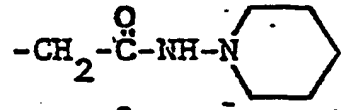
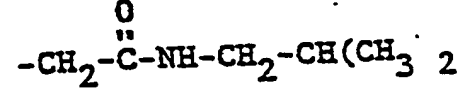
B. A solution of 60 mmoles of pyruvic acid plus 60 mmol s of L-proline ethyl ester in redistilled chloroform is cool d to -50°C in an acetone-dry ice bath. To this solution is added 60 mmoles of a precooled solution of dicyclohexyl-carbodiimide (DCC) in chloroform and the mixture is stirred at -5°C for 1 hour. The reaction mixture is slowly warmed to room temperature and stirred for an additional 2 hours and then stirred at 4°C overnight. The mixture is filtered to remove dicyclohexylurea, then cooled in an ice bath. The organic phase is washedwith cold water, cold 1N $NaHCO_3$ and finally with cold saturated NaCl. The organic phase is dried over anhydrous $MgSO_4$ and filtered. The solvent is removed with a rotary evaporator yielding N-pyruvoyl-L-proline ethyl ester.

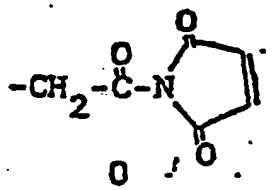
C. 40 mmoles of the product of Step A is reacted with 200 mmoles of the product of Step B in ethanol with stirring in the presence of molecular sieves at room temperature. A solution of 40 mmoles of sodium cyanoborohydride in ethan l is thenslowly added over the course of 6 hours. The reaction mixture is filtered and the solvent removed by a rotary evaporator. The product is purified by partition chromatography (Sephadex G-25), developed with butanol/acetic acid/ $H_2\ddot{O}$ (4:1:5). The ethyl esters are removed by saponification to yield the named product.

Example 3-16

By substituting any one of the reactants for propyl-amine of Example 2, and following the procedures of Exampl 2, compounds are obtained with R_1 groups as shown in the Table.

TABLE

Example	Reactant	R_1
3	butylamine	
4	cyclopentylamine	
5	L-proline	
6	adenine	
7	indoline	
8	2-azacyclooctanone	
9	aniline	
10	2-amino-5-bromo-pyridine	
11	2-amino-5-chloro-thiazole	
12	N-amino piperidine	
13	isobutyramide	

Example	Reactant	R_1
14	maleimide	
15	diacetamide	$-CH_2-\overset{O}{\overset{\|}{C}}-NH-CH_2-CH(CH_3)_2$
16	diallylamine	$-CH_2-\overset{O}{\overset{\|}{C}}-N-(CH_2CH=CH_2)_2$

Example 17

Synthesis of N-[(2-butylaminocarbonyl-1-carboxy-ethyl thio)-2-D-methylpropanoyl]-L-proline

A. 0.25 mmoles of 3-mercapto-2-D-methylpropanoyl-L-prolin ethyl ester, 0.28 mmoles of 2-bromosuccinic acid monoethyl-ester (esterified at Cl) and 0.16 mmole of K_2CO_3 were added to 0.6 ml of a 50:50 mixture of absolute ethanol and water. The suspension was stirred overnight at room temperature. 0.25 mmoles of K_2CO_3 in 0.15 ml of water was then added and the reaction mixture was stirred for an additional 24 hours. This mixture was then acidified to pH 2.0 with HCl and the product was extracted with ethyl acetate. The organic phas was washed with saturated NaCl. The product appeared to b pure and behaved as a single substance on thin layer chromatography in two separate solvent systems. The ethyl acetate phase was dried over anhydrous $MgSO_4$ and the solvent was removed with a rotary evaporator to yield the N-[3-(1-carboxyethylthio)-2-D-methylpropanoyl]-L-proline ethyl ester as a colorless oil.

B. A quantity of 0.15 mmoles of butylamine and 0.15 mmoles of the product of Synthesis A are dissolved in 0.6 ml of c ld DMF and 0.05 ml DPPA. A volume of 0.025 ml of triethylamin in DMF is added, holding the temperature at about -10°C for 2 hours. Th reaction is stored overnight at r m temperature, rotary evaporated to remove DMF, then the residue is partitioned b tween water and ethyl acetate. Th organic layer is chromatographed to obtain the nam d product.

Example 18

Synthesis of N-[1-carbethoxy-3-(methylaminocarbonyl) propyl]-D,L-Ala-L-Pro

A solution of 50 mmoles of 4-methylaminocarbonyl-2-oxo-butyric acid ethyl ester and 10 mmoles of L-Ala-L-Pro butyl ester in ethanol is stirred with powdered molecular sieves at room temperature for 30 minutes. A solution of sodium cyanoborohydride, 10 mmoles, in ethanol is added slowly over the next 5 hours. The mixture is filtered, and the solvent of the filtrate is removed with a rotary evaporator and deprotected by treatment with TFA. The product, an diethyl ester of N-[1-carbethoxy-3-methylaminocarbonyl-butyl]-alanyl-proline, is obtained after partition column chromatography [butanol/acetic acid/H_2O (4:1:5 by vol.)]

Example 19

Synthesis of N-[(1-carboxy-3-carboanilide)propyl]-δ-propyl-amido-L-glutamyl-L-pro

A. 175 mmoles of propylamine and 150 mmoles of the α-ethyl ester of Boc-glutamic acid are dissolved in 600 ml of DMF and 125 mmoles of DPPA. A volume of 25 ml of triethylamine is added drop-wise, holding the temperature at about -10°C for 2.5 hours. The reaction is stored overnight at room temperatur , rotary evaporated to remove DMF, then the product, δ-propyl-amido-L-Boc-glutamic acid ethyl ester, is saponified and then purified by chromatography on silica gel.

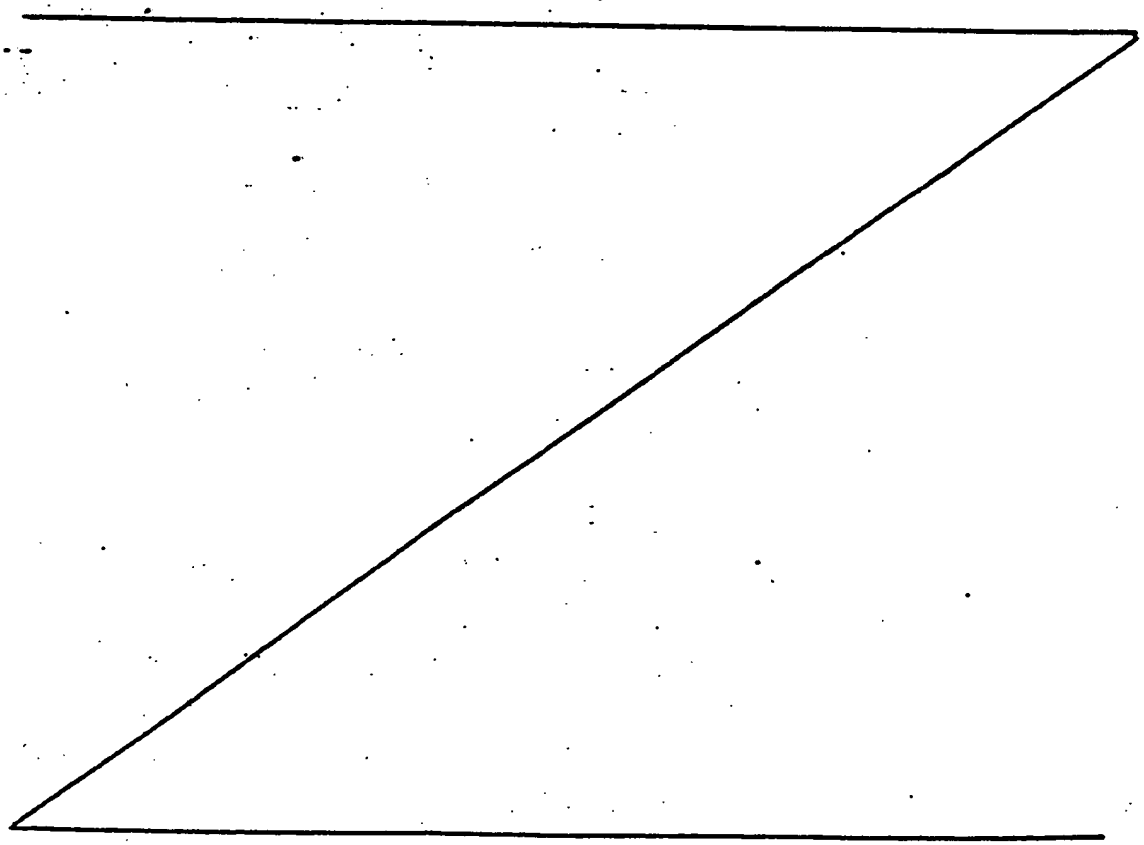
B. 100 mmoles of the product is then reacted with 100 mmol s of L-proline-t-butyl ester in redistilled dichloromethane, precooled to -5°C. To this solution is added 100 mmoles of a precooled solution of dicyclohexylcarbodiimide in dichloro-methane and the mixture is stirred in an ice bath for 2 hours. The reaction mixture is slowly warmed to room temperature and then stirred at 4°C overnight. The mixture is filtered to remove dicyclohexylurea, then cooled in an ice bath. The organic phase is washed with cold 1 N HCl cold 1N $NaHCO_3$ and finally with cold saturat d NaCl. The organic phase is dri d over an anhydrous $MgSO_4$ and filtered. The solvent is r moved with a rotary evaporator yielding δ-propylamido-Boc-L-glutamyl-L-pro-t-butyl ester. The t-butyl ester and Boc

group is removed with TFA.

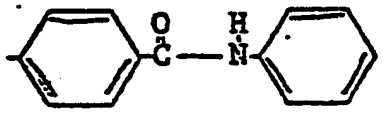
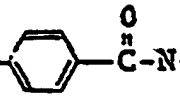
C. 50 mmoles of the product of Synthesis A of Example 9 and 250 mmoles of the product of Synthesis B of Example 19 are condensed in the presence of 50 mmoles sodium cyanoborohydride by the method described in Synthesis B of Example 1 plus 300 mmoles of $NaHCO_3$ in water to yield an esterified compound.

Examples 20-26

By substituting propylamine and the 1-ethyl ester of Boc-glutamic acid in Synthesis A of Example 19 with each pair of reactant compounds in the Table, then reacting the product with L-proline-tert-butyl ester according to Synthesis B of Example 19, and finally reacting the resulting compound with 5-carboanilide-2-ketobutyric acid ethyl ester according to Synthesis B of Example 1, a series of analogs of the product of Example 19 are obtained, which products have R_3 and R_7 groups given in the Table.



TABLE

Example	Pair of Reactants	R_7	R_3
20	methylamine, 1-ethyl ester of Boc-glutamic acid	H	$-(CH_2)_2-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{\|}{N}}-CH_3$
21	methylamine, 1-ethyl ester of Boc-2-amino-malonic acid	H	$-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{\|}{N}}-CH_3$
22	methylamine, 1-ethyl ester of Boc-aspartic acid	H	$-CH_2-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{\|}{N}}-CH_3$
23	aniline 1-ethyl ester of Boc-2-amino adipic acid	H	$-(CH_2)_3-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{\|}{N}}-$ 
24	aniline 1-ethyl ester of p-Boc-carboxy-phenylglycine	H	
25	propylamine 1-ethyl ester of p-Boc-carboxy-phenylalanine	H	$-CH_2-$  $-\overset{O}{\overset{\|}{C}}-N-CH_2-CH_2CH_3$
26	aniline 1-ethyl ester of α-Boc-methyl aspartic acid	$-CH_3$;	$-CH_2-\overset{O}{\overset{\|}{C}}-\overset{H}{\underset{\|}{N}}-$ 

Example 27

Preparation of N-[L-1-benzyloxycarbonyl-3-(carbo-4-iodoanilide)propyl]-Alanyl-L-Proline

A. Synthesis of L-glutamic acid-α-benzyl ester-γ-4-iodoanilide.

A solution of 4 mmoles of N^{α}-Boc-L-Glu-γ-2-NO_2-phenyl ester-α-benzyl ester in 3 ml of CH_2Cl_2 was added to a solution of 4.1 mmoles of 4-iodo-anilin in 3 ml of CH_2Cl_2 and the resulting solution was stirred at r om temperature ov rnight. (The reaction was judged to be c mplete by thin layer chromatography). An oily residue was btained

after work-up. The product was dissolved in 4 ml of anhydrous trifluoroacetic acid. After 45 minutes at room temperature, the solvent was removed by rotary evaporation at 40°C. White crystals were formed after the addition of 4.5 ml M HCl in ethyl acetate. The mixture was left at 0°C for one hour and was filtered. The precipitate was washed with cold ethyl acetate in ether and then dried over P_2O_5 and NaOH in a vacuum desiccator. Yield 0.79 g; d.p. 119-120°C; second crop yield 1.11 g; d.p. 119-120.5°C. The material was recrystallized from $CHCl_3$/isopropyl ether; d.p. 119.5 - 120.5°C. Elemental analysis for $C_{18}H_{10}N_2IClO_3$: Calculated C 45.54; H 4.24; N 5.90; I 26.73 Cl 7.47; O 10.11. Found: C 45.55; H 4.19; N 5.92; I 26.53; Cl 7.34.

B. Synthesis of N-L-[1-benzyloxycarbonyl-3-(carbo-4-iodo-anilide)propyl]-Ala-L-Pro-t-butyl ester

A solution of the product of A (1 mmole in 1 ml of ethanol) is added with stirring to 1 mmole of $NaHCO_3$ in 0.2 ml of H_2O. To the resulting solution was added 5 mmoles of N-pyruvoyl-L-proline-t-butyl ester in 2 ml of ethanol plus 1.6 g of molecular sieves. The mixture was stirred for 30 minutes at room temperature. Sodium cyanoborohydride, 65 mg in 1.5 ml of ethanol, was added, drop-wise, over a period of 4 hours. The reaction mixture was left at room temperature overnight. The mixture was filtered, the filtrate saved, and the precipitate was washed several times with ethanol. Solvent of the combined filtrates was removed by rotary evaporation at 30°C to yield a yellow oil. The crude product was purified on Sephadex LH-20 (2.22 x 99 cm column) developed with THF/isopropanol (3:7 by vol.); 250 drops (5.8 ml/fraction). Fractions 33-35 contained the desired product.

C. The named compound of this Example was obtained by dissolving the desired product of B in 2 ml of anhydrous TFA. The solution was allowed to stand at room temperature for 30 minutes and then the trifluoroacetic acid was removed by rotary evaporation at 30°C. The residue was dissolved in a small amount of ethanol, and the solution was applied to a column (1.2 x 43 cm) of AG1-X2 (OH^- form) in H_2O. The column was developed with H_2O, 62 ml, and then a linear

gradient was developed between H_2O and 0.5M ammonium acetate (2 liters total). The column was washed with 0.5M ammonium acetate (1 liter), 1.0M ammonium acetate (200 ml) and then with 1.0M ammonium acetate/ethanol (1:2 by vol). The desired product was eluted with the last-named solution. Solvent volume was reduced by rotary evaporation and then ammonium acetate was removed by lyophilization and sublimation.

Example 28

Preparation of N-[L-1-carboxy-3-(carbo-anilide) propyl]-Alanyl-L-Proline

The product of Example 27, 40 mg, in 3 ml of methanol, was reacted with 20 mg of 10% palladium on carbon and H_2 at 1 atmosphere for 3 hours at room temperature. The precipitate was removed by filtration, and the solvent of the filtrate was removed by rotary evaporation. The desired product was obtained by chromatography on Sephadex G-10 (1.2 x 96 cm column) developed and eluted with 2% pyridine in water (yield 12.1 mg).

Example 29

Preparation of N-[L-1-carboxy-3-(carbo-4-iodo-anilide) propyl Alanyl-L-Proline

The product of Example 27, 60 mg, was treated with 3 ml of anhydrous HF in the presence of anisole for 1 hr. The desired product was obtained by the chromatographic system of Example 28. Yield 22.15 mg.

Example 30

Preparation of N-[L-1-carboxy-2-(carbopyrrolide)ethyl]-Alanyl-L-Proline.

A. Synthesis of L-aspartic acid-β-pyrrolide-α-ethyl ester.

N^{α}-Cbo-L-aspartic acid-α-ethyl ester, 8 mmoles, in 5 ml of CH_2Cl_2 was cooled to -5°C. A cold solution of DCC, 8 mmoles in 3 ml of CH_2Cl_2 was added with stirring. To this solution was added 0.67 ml of pyrrolidine. Stirring was continued at -5°C for 30 minutes and at 4°C overnight. The mixture was filtered, and the precipitate was washed with ethyl acetate. The combined filtrate was washed until neutral. The organic phase was dried over $MgSO_4$ and then filtered. The solvent of the filtrate was removed under

vacuum to yield 1.85 of a yellow oil. The oil, 1.5 g, was dissolved in 20 ml of methanol and the Cb-protecting group was removed by hydrogenolysis (150 mg of 10% palladium on carbon with H_2 at 10 pounds per square inch for 90 minutes). The mixture was filtered, and solvent was removed under vacuum to yield white crystals. Recrystallization was effected from methanol/isopropyl ether. The desired product remained in the mother liquid and was converted to its HCl salt by adding HCl in ethyl acetate. Solvent was removed and the residue was dried over P_2O_5 and KOH in a vacuum desiccator to yield a hydroscopic foam. Crystals, 0.47 g, were obtained from $CHCl_3$/ethyl acetate.

B. Alkylation of pyruvoyl-L-proline with the product of A (Example 30)

Molecular sieves (1.312 g) were added with stirring to a mixture of 0.206 g of HCl·L-Asp-$^{\beta}$-pyrrolide-α-ethyl ester, 0.073 g of $NaHCO_3$ and 0.986 g of N-pyruvoyl-L-proline-t-butyl ester in 0.1 ml of H_2O and 2.0 ml of ethanol at room temperature. The mixture was stirred for 30 minutes and then 0.054 g of sodium cyanoborohydride in 1.0 ml of ethanol was added drop-wise over a period of 4 hours. Stirring was continued for another 18 hours. The mixture was filtered and the precipitate was washed with ethanol. Solvent of the combined filtrates was removed by rotary evaporation to yield a yellow oil. The material was chromatographed on LH-20 (2.2 x 99 cm) developed with THF/isopropanol (3:7 by vol). The residue obtained by rotary evaporation was dissolved in 1.2 ml of TFA. After 45 minutes at room temperature, TFA was removed and the material was purified by chromatography on AG1-X2 (1.2 x 38 cm) developed first with H_2O and then with a linear gradient between H_2O and 0.5M ammonium acetate. Apparently pure product, 31.5 mg, was obtained by chromatography on Sephadex G-10 (1.2 c 97 cm column) developed with 2% pyridine. The ethoxy group was removed by saponification.

Example 31

In vitro assays of the potency of selected compounds as inhibitors of angiotensin converting enzyme.

Compounds of this invention were assayed through the following protocol: 25 microliters of buffer (0.05M Hepes buffer, pH 8.0, plus 0.3 M NaCl and 0.75 M Na_2SO_4) or 25 microliters of an inhibitor in buffer was added to the bottom of a 7 ml liquid scintillation vial. To this was added 100 microliters of buffered substrate [S], [^{3}H]benzoyl-Gly-His-Leu, 80 nM (25 Ci/mmole). The reaction was started by adding 100 microliters of partially-purified human plasma angiotensin converting enzyme, or 100 microliters of buffer alone. The concentration of enzyme [E] used was that required to hydrolyze 8-12% of substrate when incubated at 37°C for 15 minutes. The scintillation vials and their contents were incubated at 37°C for 15 minutes, and the reactions were stopped by adding 200 microliters of 0.5 M HCl to each vial. The radioactive reaction product, [^{3}H]benzoyl-Gly(hippuric acid), was separated from unhydrolyzed substrate by adding and mixing (by inversion) 3 ml of Ventrex Cocktail No. 1 (Ventrex Laboratories, Inc., Portland, Main), a fluid disclosed in copending U.S. patent application No. 184,653, filed September 6, 1980. Extractable ^{3}H was quantified by liquid scintillation counting. Substrate, in c.p.m., was quantified by scintillation counting of a vial containing 100µl of buffered substrate in 5 ml of RIAfluor (New England Nuclear). The reaction mixture containing all constituents except for inhibitor was termed the control (C). The reaction mixture lacking enzyme and inhibitor was called the blank (B). Reaction mixtures containing inhibitor (varied over the range of 10^{-4} - 10^{-12}M) were called the test (T) reactions. Under the conditions of this assay, th reaction obeys first order enzyme kinetics, thus the concentration of inhibitor required to inhibit th rat of hydrolysis by half (I_{50}) approximat the Ki value. The results were estimat d by us of the formula:

$$[E] = \frac{C - B}{[S]} \times 100 \times \frac{1}{15 \text{ min}}$$

where C = control c.p.m.; B = blank c.p.m.; [S] = substrate c.p.m. The factor 100 converts fractional substrate utilization into percentage utilization, and 1/15 minute connects to percentage substrate utilization/minute. Thus, [E] is enzyme activity in percentage substrate utilization/minute. By substituting T for C, hydrolysis rates are computed for the test reaction mixtures. By comparing a given test rate against the control rate, the degree of inhibition can be computed.

Compound Product of Example No.	I_{50}
28	5.2×10^{-9} M
29	2.3×10^{-10} M
30	1.4×10^{-7} M

Example 32

Intravenous effectiveness of N-[L-1-carboxy-3-(carbo-4-iodoanilide)propyl]-D,L-Ala-L-Pro.

Rats (190 - 290 g body weight) were fasted overnight and then anesthetized with intraperitoneal pentobarbital, 50-60 mg/kg. Tracheostomy was performed and the animals were ventilated mechanically. A cannula was inserted into a femoral vein for injection of angiotensin I, and a second cannula was inserted into a common carotid artery for direct measurement of arterial blood pressure. Heparin, 1,000 units, was injected via the femoral vein to prevent coagulation. Blood pressure was measured with a pressure transducer connected to a polygraph. The rats were injected with 400 mg/kg of angiotensin I in 20µl of 0.9 g % NaCl, an amount of angiotensin I sufficient to raise mean arterial blood pressure by approximately 48 mm Hg.

After the responsiveness of a given rat to angiotensin I was established, the named compound at 0.5 micromole/kg (drug dissolved in 15 microliter of 0.9% NaCl) was given intravenously. At timed intervals, the effects of 400 ng/kg of angiotensin I on mean arterial blood pressure were tested.

Results are shown below:

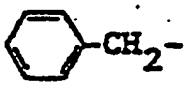
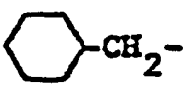
Time after IV Administration (minutes)	Blood Pressure Response to 400 ng/kg of Angiotensin I (% of Control)
-5	100% (48 mm hG.)
+1	33%
5	42%
10	46%
15	52%
20	60%
25	67%
30	71%
35	71%
50	83%
60	92%
70	100%
80 min.	104%

Example 33

"Intravenous Effectiveness of N-[L-1-carboxy-3-(carboanilide)propyl]-D,L-Ala-L-Pro"

Experiments were carried out using rats according t Example 32. The results are shown below:

TABLE

Example	α-keto carboxylic acid	R_3
34	pyruvic acid	CH_3-
35	phenylpyruvic acid	
36	3-cyclohexyl-2-oxo-propionic acid	
37	6-methyl-2-oxo-heptanoic acid	

S-benzyl-cysteine-t-butyl est r
S-benzyl-hom cy tein -t-butyl ster
S-methyl-h m cy tein ethyl ester
S-ethyl-homocysteine-ethyl ester
S-t-butyl-homocysteine-t-butyl ester
O-t-butyl-homoserine-t-butyl ester
O-benzyl-homoserine-benzyl ester
O-methyl-homoserine-methyl ester
O-ethyl-homoserine-ethyl ester
O-phenyl-homoserine-ethyl ester
O-phenyl-serine-ethyl ester
S-phenyl-cysteine-ethyl ester
β-fluoro-phenylalanine ethyl ester
β-OH-phenylalanine-methyl ester
β-Br-alanine-methyl ester
β-thienylserine-t-butyl ester
3,5-dimethyl-tyrosine-t-butyl ester
β-hydroxynorvaline ethyl ester
β-benzyloxynorvaline-ethyl ester
N^{ε}-Boc-hydroxylysine t-butyl ester
3-Boc-amino-tyrosine-ethyl ester
α-methyl-phenylalanine-ethyl ester
t-leucine methyl ester
α-methyl glutamine methyl ester
N^{ε}-hydroxylysine t-butyl ester
β-N-methyl-lysine-methyl ester
5,5'-dihydroxy-leucine-ethyl ester
β-fluoro-asparagine-ethyl ester
β-methyl-asparagine-ethyl ester
γ-N-methyl-lysine-methyl ester
β-methyl-β-benzylamido-aspartic acid-β-ethyl ester
2-ethoxy-5-NO_2-phenylalanine ethyl ester
β-ethoxy-phenylalanine-t-butyl ester
α-methyl-serine-t-butyl ester
O-benzyl-α-methyl-serine-t-butyl ester
O-benzyl-α-methyl-s rine-t-butyl ester

C. Any f the α-amides or α-imid s f D-cysteine-α-benzyl e t r of Synthesis B of this example ar then us d to alkylate any of the α -keto arboxylic acids in Table II, immediately b low. A quantity f 5 mmoles f any f the α-amides or α-imides of D-cysteine-α-benzyl ester of Synthesis B is dissolved in 1 ml of ethanol and added with stirring to 5 mmoles of $NaHCO_3$ in 0.2 ml H_2O. To the resulting solution is added 25 mmoles of any α-keto carboxylic acid of Table II in 2 ml of ethanol plus 1.6 g of molecular sieves. The mixture is stirred for 1 hour at room temperature, then 5 mmoles of sodium cyanoborohydride, in 1.5 ml of ethanol, i added drop-wise over a period of 4 hrs. The reaction mixture is left at room temperature overnight. After filtration, solvent is removed from the filtrate, and the product purified by column chromatography. The products are compounds of the formula:

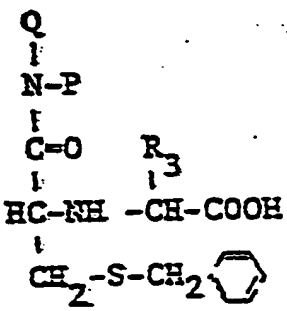
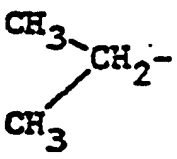
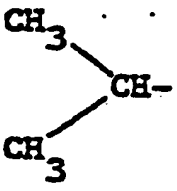
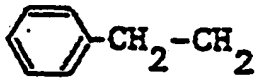
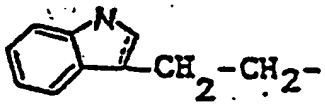
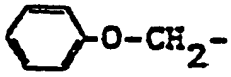
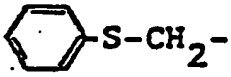
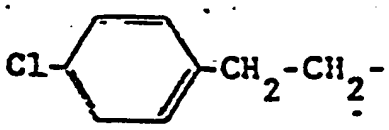


TABLE II: α KETO CARBOXYLIC ACIDS

pyruvic acid
phenylpyruvic acid
3-cyclohexyl-2-oxopropionic acid (cyclohexylpyruvic acid)
6-methyl-2-oxoheptanoic acid
4-methyl-2-oxopentanoic acid
2-oxobutyric acid
3-methyl-2-oxobutyric acid
2-oxoglutanic acid
2-oxoadipic acid
2-oxo-4-phenylbutyric acid (and its t-butyl ester)
4-(3-indolyl)-2-oxobutyric acid
N-acetylamin ethyl-2-oxo-4-ph nylbutyrat
dimethylaminoethyl-2-oxo-4-phenylbutyrate

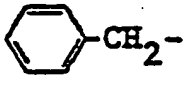
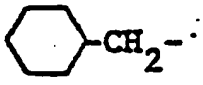
Example	α-keto carboxylic acid	R_3
38	4-methyl-2-oxo-pentanoic acid	
39	2-oxo-butyric acid	CH_3CH_2
40	3-methyl-2-oxo-butyric acid	
41	2-oxo-glutaric acid	$EtOOC-CH_2-CH_2-$
42	2-oxo-adipic acid	$EtOOC-CH_2CH_2CH_2-$
43	2-oxo-4-phenyl butyric acid	
44	4-(3-indolyl)-2-oxo-butyric acid	
45	phenoxypyruvic acid	
46	phenylthio pyruvic acid	
47	4-p-chlorophenyl-2-oxo-butyric acid	

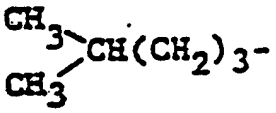
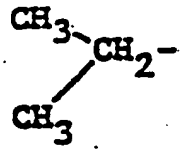
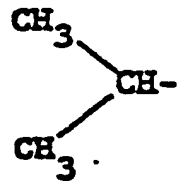
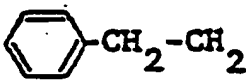
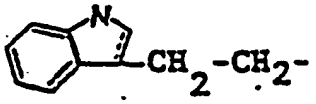
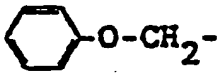
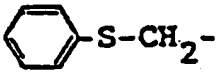
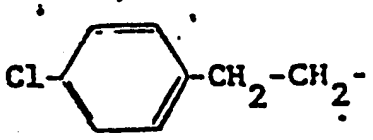
Time After IV Administration (minutes)	Blood Pressure Reponse to 400 ng/kg of Angiotensin I (% of Control)
-5	100% (48 mm Hg.)
+1	42%
5	37%
10	37%
15	40%
20	40%
25	40%
30	35%
40	35%
50	46%
60	46%
70	48%
80	52%
90	54%
102 min.	62.5%

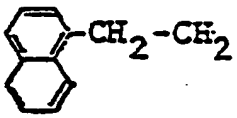
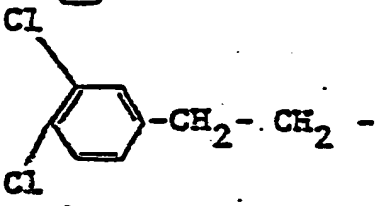
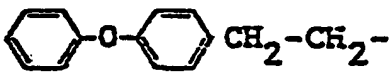
Examples 34-52

By substituting pyruvic acid in Synthesis B of Example 2 with any of the α-keto carboxylic acids (appropriately protected) in the Table, and reacting the product with 3-(propyl amino-carbonyl)-2-amino-propanoic acid ethyl ester as in Synthesis A of Example 2, products with R_3 groups in the Table are formed.

Table

Example	α-keto carboxylic acid	R_3
34	pyruvic acid	CH_3-
35	phenylpyruvic acid	
36	3-cyclhexyl-2-oxo-propionic acid	

Example	α-keto carb xylic acid	R_3
37	6-methyl-2-oxo-heptanoic acid	
38	4-methyl-2-oxo-pentanoic acid	
39	2-oxo-butyric acid	CH_3CH_2
40	3-methyl-2-oxo-butyric acid	
41	2-oxo-glutaric acid	$EtOOC-CH_2-CH_2-$
42	2-oxo-adipic acid	$EtOOC-CH_2CH_2CH_2-$
43	2-oxo-4-phenyl butyric acid	
44	4-(3-indolyl)-2-oxo-butyric acid	
45	phenoxypyruvic acid	
46	phenylthio pyruvic acid	
47	4-p-chlorophenyl-2-oxo-butyric acid	

Example	α-keto carboxylic acid	R_3
48	indole-3-pyruvic acid	
49	2-oxo-3-p-cyanophenyl-propionic acid	
50	4-alpha-naphthyl-2-oxo-butyric acid	
51	4-(3,4-dichlorophenyl)-2-oxo-butyric acid	
52	2-oxo-4-p-phenoxy-phenyl butyric acid	

Example 53

Synthesis of 2-keto-butyryl-L- ⟨Glu.

L-pyroglutamic acid (35 mmoles) is suspended in a mixture of 35 ml of propylene oxide and 210 ml of dry acetonitrile at room temperature. Bis-trimethylsilyl-tri-fluoro acetamide (77 mmole) is added and the stopped reaction is stirred at room temperature for 15 minutes. 2-keto-butyric acid mixed carbonic anhydride (prepared by 2-keto-butyric acid, 36.8 mmole, triethylamine in iso-butyl chloro formate) is added and the reaction is stirred at room temperature overnight. Acetonitrile is then removed in vacuo and the resulting residue is dissolved in ethyl acetate. The organic phase is washed with H_2O, then saturated NaCl, dried over anhydrous Na_2SO_4 and filtered, and the solvent removed with a rotary evaporator.

B. Synthesis of N-2-(1-anilinocarbonyl-2-benzylthioethyl)-butyryl-L-pyroglutamic acid.

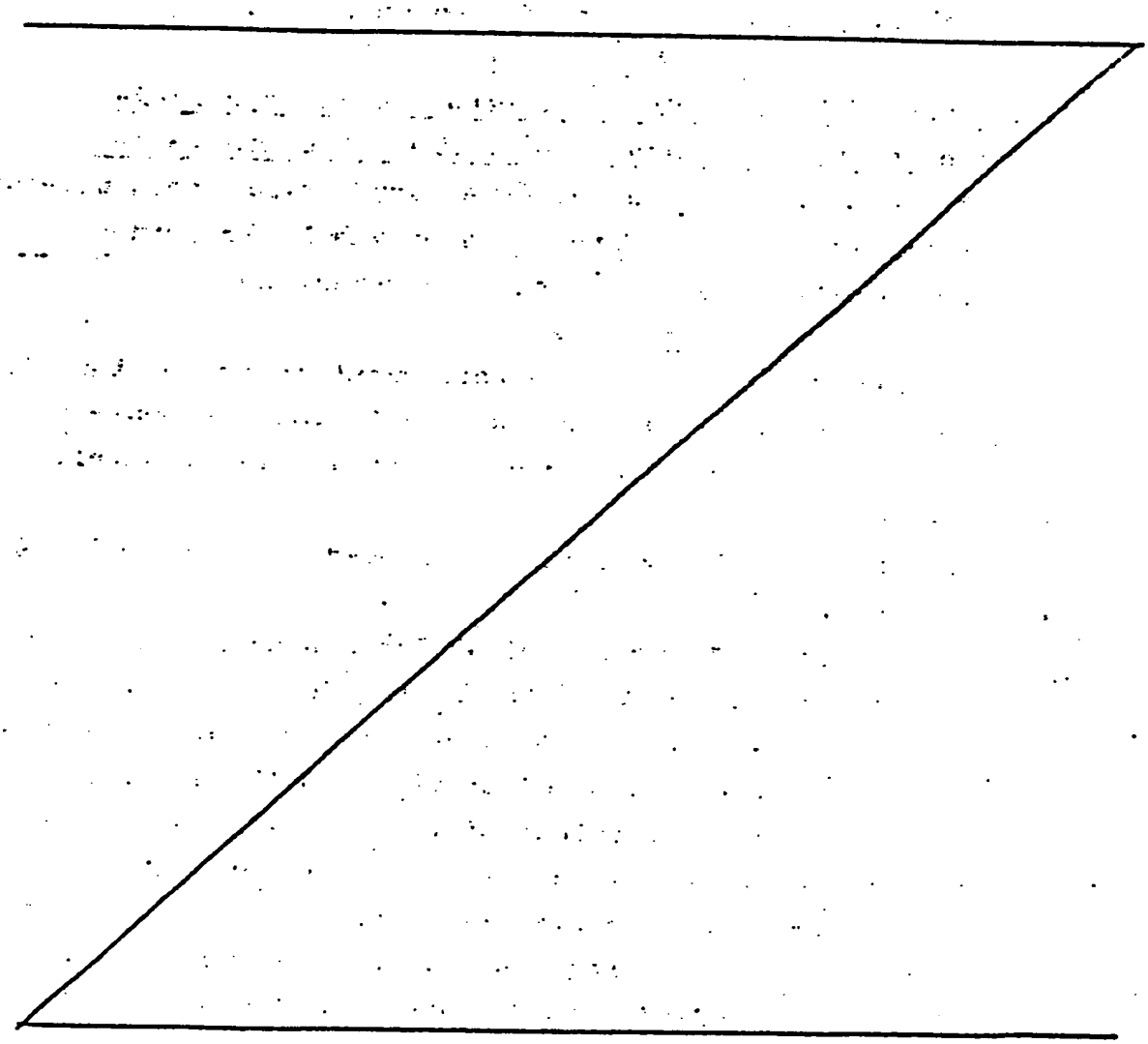
Nα-Boc-S-benzyl-D-cysteine 100 mmols is reacted with an equivalent of aniline in the presence of a slight xcess

f on equival nt of DCC (105 mmoles) by th method describ d in Synthesi B f Example 2. The Boc group is th n r moved with anhydrous TFA. 50 mmole f the pr duct is then coupled t 10 mmoles 2-keto-butyryl-L-<Glu (Synthesis A) with 10 mmoles of cyanoborohydride according to Example 18 to yield the named compound.

Example 54

Synthesis of N-2-(1-anilinocarbonyl-2-benzylthioethyl)-propanoyl-L-pro.

By substituting pyruvoyl-L-pro (Example 2) for 2-keto-butyryl-L-<Glu (Synthesis A of Example 53), and following the procedure of Synthesis B of Example 53, the named product is obtained.



Exampl 55

Synthesis of N-[-1-(N-acetylaminoethoxy carbonyl)-3-(carboanilid) propyl]-D,L-Ala-L-Pro-ethyl ester.

A solution of 50 mmoles of 2-Boc-amino-4-carboxy butyric acid N-acetyl aminoethyl ester is coupled to 50 mmoles of aniline in the presence of an equivalent of DCC according to Synthesis B of Example 2. The Boc group is then removed with anhydrous TFA. 40 mmoles of the product is then reacted with 200 mmoles of N-pyruvoyl-L-Pro-ethyl ester, then 42 mmoles of sodium cyanoborohydride in ethanol is slowly added over the course of 6 hours. The reaction mixture is filtered and the solvent removed by a rotary evaporator, yielding the named compound.

Example 56

Synthesis of N-[L-1-(dimethylaminoethoxycarbonyl)-3-(carbonylmethylamino)propyl]-D.L-Ala-L-Pro ethylester.

By substituting 2-amino-4-carboxy butyric acid dim thyl aminoethyl ester for 2-amino-4-carboxy butyric acid of Example 55, the named compound is synthesized.

Example 57

A. By substituting L-proline-tert butyl ester for L-prolin ethylester in Synthesis B of Example 2, the procedure of Synthesis B of Example 2 yields the pyruvoyl-L-Pro-tert-butyl ester.

The tert butyl ester can be removed by treatment with TFA in anisole.

B. A solution of 10 mmoles of N^{α}-Boc-S-benzyl-D-cysteine-α-NO_2-phenyl ester in 3 ml of CH_2Cl_2 is added to a solution of 10.5 mmoles of any of the amine or imine compounds (listed in Table I, below) in 3 ml of CH_2Cl_2, and the resulting solution is stirred overnight at room temperature. The reaction is judged to be complete by thin layer chromatography. The resulting mixture is dissolved in 4 ml of TFA to remove the Boc blocking group, rotary evaporated and crystallized, to yield α-amides and α-imid s of D-cystein - α-benzyl ester as the product.

TABLE I: AMINE AND IMINE COMPOUNDS

aniline
benzylamin
methylamine
ethylamine
1-aminopropane
2-aminopropane
2-aminobutane
1-amino-2-butanone
t-butylamine
cyclopentylamide
cyclohexylamine
ε-aminocapric acid benzyl ester
ε-aminocaproamide
3-amino-2-methyl-propionic acid ethyl ester
2-amino-propionic acid ethyl ester
glycine-t-butyl ester
valine-benzyl ester
p-OH-aniline
p-OH-m-iodo-aniline
p-carboxy-thienyl ester of aniline
m-F-benzylamine
4-OH-3,3'-Br-benzylamine
4-Cl-benzylamine
3,4-dichloro-benzylamine
3-NO_2-benzylamine
3-phenylpropylamine
2-indolylethylamine
2-amino-pyridine
adenine
Cytidine
pyrroline
4-phenylbutylamine
α-methyl-alanine ethyl ester
3-hydroxy-propylamine
3-Boc-amino-propylamine
1-amino-3-hydroxy-butane
1-adamantanamine

2-adamantanamin
1-adamantanemethyl amin
N^{ε} -8 c-lysine-ethyl ester
N^{α} -Boc-lysine-t-butyl ester
N^{ϑ} hydroxy-arginine-ethyl ester
N^{ϑ} methyl-homoarginine-t-butyl ester
N^{im}-benzyl-histidine-t-butyl ester
leucine-t-butyl ester
isoleucine-t-butyl ester
norvaline-ethyl ester
norleucine-methyl ester
glucine-p-methyl benzyl ester
α-methyl-alanine-diphenylmethyl ester
glycyl-benzylamide
α-methyl-alanyl-4-OH-benzylamide
N^{im}-benzyl-histidinyl-3-iodo-anilide
glycyl-pyrrolide
glycyl-1-adamantanamide
glutamine-ethyl ester
asparagine-t-butyl ester
α-methyl-valine-t-butyl ester
α-methyl-phenylalanine-t-butyl ester
tyrosine-t-butyl ester
O-benzyl-tyrosine-t-butyl ester
4-iodo-phenylalanine ethyl ester
3,5-dibromotyrosine-ethyl ester
thyronine-ethyl ester
vinyl glycine ethyl ester
β-fluoro-alanine ethyl ester
serine ethyl ester
threonine t-butyl ester
O-t-butyl-threonine-t-butyl ester
O-t-butyl-serine-ethyl ester
O-benzyl-serine-ethyl ester
O-methyl-serine-methyl ster
O-ethyl-serine- thyl ester
S-ethyl-cystein -ethyl ester
S-t-butyl-cysteine-t-butyl ester

2-oxo-5-methylhexonic acid
phenoxypyruvic acid
phenylthi pyruvic acid
4-p-chloroph nyl-2-ox butyric acid
indole-3-pyruvic acid
2-oxo-3-p-cyanophenylpropionic acid
4-α-naphthyl-2-oxobutyric acid
4-(3,4-dichlorophenyl)-2-oxo-butyric acid
2-oxo-4-p-phenoxyphenylbutyric acid

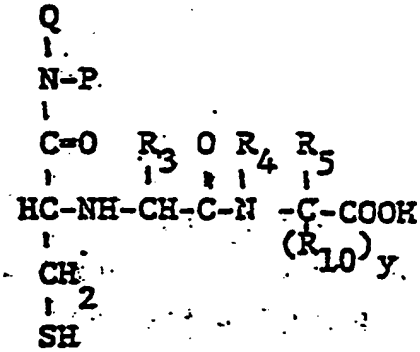
D. Any of the products of synthesis C of this example ar reacted with L-proline ethyl ester, or L-proline-tert butyl ester or an α-ethyl ester of any of the L-proline analogs listed in Table III, immediately below. The reaction is carried out according to the coupling procedures of Synthesis B in Example 2 or Synthesis A in Example 53.

Table III

3,4-dehydroproline
2,3-dehydroproline, 4,5-dehydroproline
2-OH-proline
3,4-di-OH-proline
3-methoxyproline
2-methoxyproline
3,4-dimethoxy proline
4-fluoro-proline
3-fluoro-proline
2-fluoro-proline3,4-di-OH-proline
3-methoxyproline
2-methoxyproline
3,4-dimethoxyproline
4-fluoro-proline
3-fluoro-proline
2-fluoro-proline
3,4-fluoroproline
2,3-difluoro-proline
3,4-difluoro-proline
4-Cl-proline
3-Cl-proline
2-Cl-proline
3,4-dichloro-proline

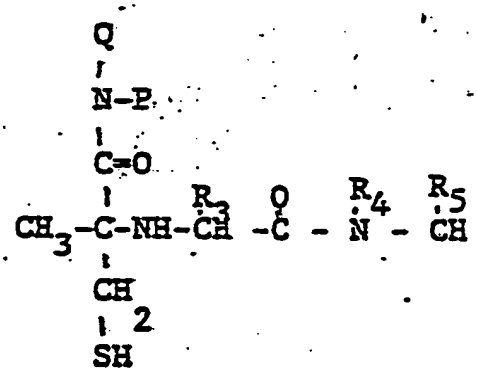
2,3-dichloro-proline
3-Br-proline
2-Br-proline
3,4-dibromo-proline
2,3-dibromo-proline
4-iodo-proline
3-iodo-proline
2-iodo-proline
3,4-diiodo-proline
5-phenyl-thioproline
5-hydroxyphenyl-thioproline
(o-, m- or p-)
4-mercapto-proline-proline
3-mercapto-proline
4-methylthio-proline
3-methylthio-proline
4-aminomethyl-proline
3-aminomethyl-proline
β -thioproline
α -methyl-proline
3-OH-5-methyl-proline
4-methylene proline
4-hydroxymethyl-proline
4-propyl-proline
3-propyl-proline
L-proline
L-pyroglutamic acid
4-Keto-L-proline
3-Keto-L-proline
4-hydroxy-L-proline
3-hydroxy-L-proline
L-pipecolic acid
4-methoxy-L-proline
4-bromo-L-proline
L-thiazolidine-4-carboxylic acid
5-Ketoproline
L-2-azetidine carboxylic acid

Products of Synthesis D of this example are saponified to remove the ethyl ester. They are treated with anhydrous HF in the presence of anisol to remove the S-benzyl protecting group. If the ethyl ester or benzyl ester group are removed, the final product of Synthesis D of this example has the formula:



Example 58

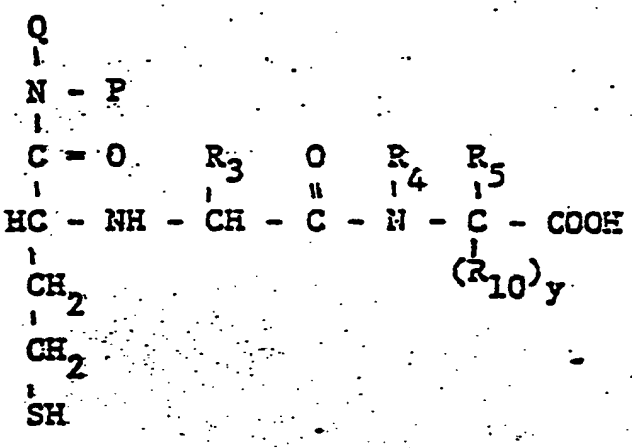
By substituting the N^{α}-Boc-α-methyl-S-benzyl-D-cysteine-α-NO_2-phenyl ester for N^{α}-Boc-S-benzyl-D-cysteine-α-NO_2-phenyl ester of Synthesis B of Example 57, and following the procedures of Example 57, compounds of the formula:



are isolated.

Example 59

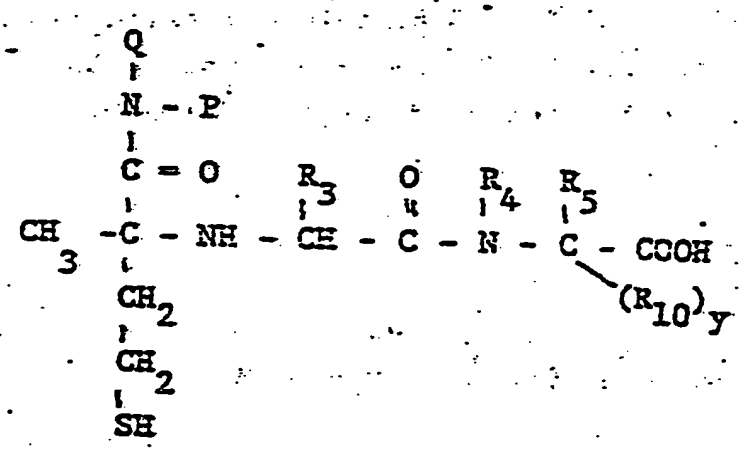
By substituting N^{α}-Boc-S-benzyl-D-homocysteine-α-NO_2-phenyl ester for N^{α}-Boc-S-benzyl-D-cysteine-α-NO_2-phenyl ester of Synthesis B of Example 57, and following the procedures of Example 57, compounds of the formula



are obtained.

Example 60

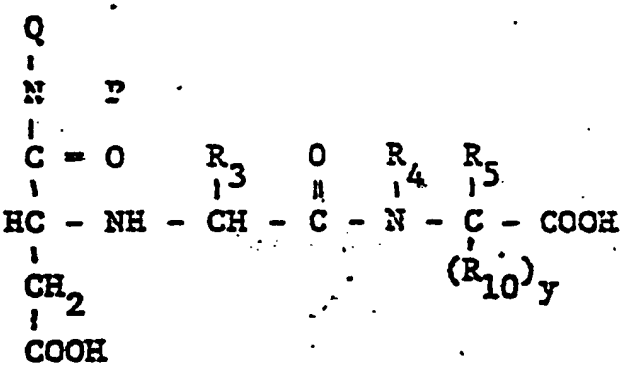
By substituting N^{α} -Boc - S - benzyl - α-methyl - D - homocysteine - α - NO_2 phenyl ester for N^{α} - Boc - S - benzyl - D - cysteine - α - NO_2 - phenyl ester of Synthesis B in Example 57, and following the procedures of Example 57, compounds of the formula:



are obtained.

Example 61

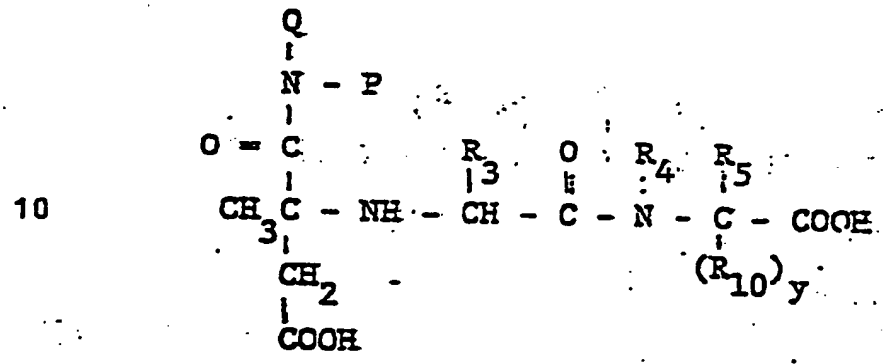
By substituting N^{α} - Boc - D aspartic acid - α - NO_2 - phenyl ester - β - ethyl ester for N^{α} - Boc - S - benzyl - D - cysteine - α - NO_2 - phenyl ester of Synthesis B in Example 57, and following the procedure of Example 57, with selected deprotection steps, compounds of the formula:



are obtained.

Example 62

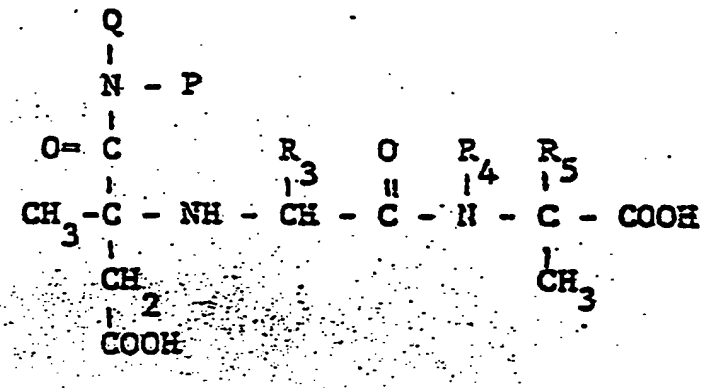
By substituting N^{α} - Boc - α - methyl - D - aspartic acid - α - NO_2 - phenyl ester - β - ethyl ester for N^{α} - Boc - S - benzyl - D - cysteine - α - NO_2 - phenyl ester of Synthesis B in Example 57, and following the procedures f Example 57, with selected deprotection steps, compounds f the formula



are obtained.

Example 63

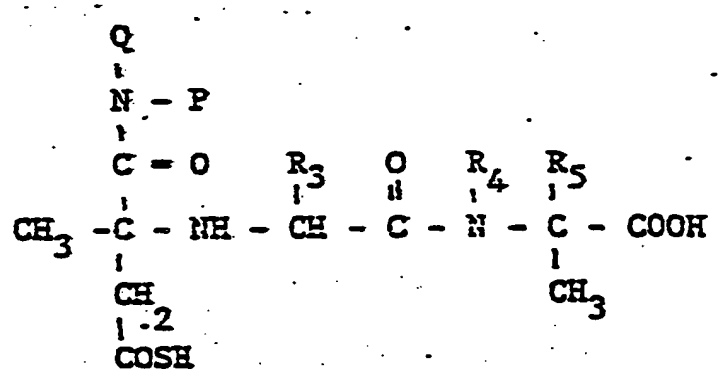
By substituting N^{α} - Boc - α - methyl- D - aspartic - α - p - NO_2 - phenyl ester - β - ethyl ester for N^{α} - Boc - S - benzyl - D cysteine - α - NO_2 - phenyl ester of Synthesis B in Example 57, and by adding N-pyruvoyl - α - methyl - L - prolin - t - butyl ester as the 2-keto - acyl - proline ester analog in Example 57, and foll wing the procedures f Example 57, with selected deprotection st ps, compounds of th formula:



are obtained.

Example 64

By substituting N^{α} - Boc - α - methyl - D - aspartic - α - NO_2 - phenyl ester - β - thiophenol ester for N^{α} - Boc - S - benzyl - D - cysteine - α - NO_2 - phenyl ester of Synthesis B in Example 57, and following the procedures of Example 57 with selected deprotection steps, including treatment with NaSH, compounds of the formula:



are obtained.

The foregoing examples are intended to be illustrative, not limiting. Many other variations of the present invention will readily occur to those of ordinary skill in the art, and it is intended that such variations are within the scope of the invention and the appended claims.

CARPMAELS & RANSFORD

CHARTERED PATENT AGENTS · EUROPEAN PATENT ATTORNEYS · TRADE MARK AGENTS

DEREK O. R. GRUNDY
JOHN R DAVY, M.I.E.M.A. IAN S.P de M.DEVALIX, M.A.(CANTAB)
S.DAVID VOTIER, B.Sc. JOHN W. N. CARPMAEL
ALAN J.JONES, B.Sc. STEPHEN J.COLGAN, Ph.D.

W. B. A. DAVIES, M.A. (OXON) B.V. INGRAM, M.Inst.P.
M. J. DONNAN, B.Sc. R. M. JOHNSTON, B.Sc.
N. R. HOWICK, M.A. (OXON)

R. O. SAWTELL, (MANAGING CLERK)

CONSULTANTS:
MICHAEL J. HOOLAHAN, B.Sc.
W. PHILIP WILLIAMS, M.A.(CANTAB) MAURICE H. CARPMAEL

43 BLOOMSBURY SQUARE
LONDON · WC1A 2RA

TELEPHONE: 01-242 8692 AND 7731
TELEX: 267209
RANK XEROX TELECOPIER 400
AND AT
15 SOUTHGATE STREET, WINCHESTER, HANTS SO23 9DZ
TELEPHONE: 0962 68656
AND
ZEPPELINSTRASSE 71, 8 MUNICH 8
PLEASE REPLY TO LONDON OFFICE

YOUR REF

OUR REF IBMD/JB

29 November, 1982

The European Patent Office,
Receiving Section,
P.B. 5818 Patentlaan, 2
2280 HV Rijswijk (ZH)
Netherlands.

Registered

The request for correction is allowed under R. 88 EPC / with the exception of the deleted points /.
THE HAGUE, 07.12.82
RECEIVING SECTION

Dear Sirs,

European Patent Application 82304377.3
University of Miami.
Examination as to formal requirements - invitation to remedy deficiencies
(Rule 41, paragraph 1 EPC)

In accordance with your letter of 7 September, 1982 under the above heading, I enclose three copies of a retyped description and claim 1.

Please note that in the retyping the opportunity was taken to correct the following clerical errors (the references are t the pages of the original specification).

Page 5, line 18 - "bnding" changed to "binding"

Page 17 , lines 4 and 6 - double "y" taken out of "hydroxyyphenyl" and "alkylcarbonyloxyyphenyl"

Page 17, line 29 - "z" changed to "Z"

Page 20, line 11 -"(k)" changed to "(i)"

Page 38, lines 6 and 7 - "l" inserted in the middle of"dimethyhydantoin" and "-diethyethylenediamine"

Page 67, line 15 - "]" changed to "["

Claims page 3, sixth lin from bottom - "s" tak n off "carbons"

Pag 4 in VII - "s" tak n off "groups"

Page 11, line 10 from bottom - "y" put in "hydroxphenyl".

cont..

In the originally-filed specification there were manuscript amendments on pages 66 and 67 (Examples 53 - 56) correcting "isobutyric" to "butyric". These amendments hav , of course, been preserved in the retyped specification.

Please acknowledge receipt of this letter and its enclosur s by signing or stamping the enclosed copy letter and returning it to me.

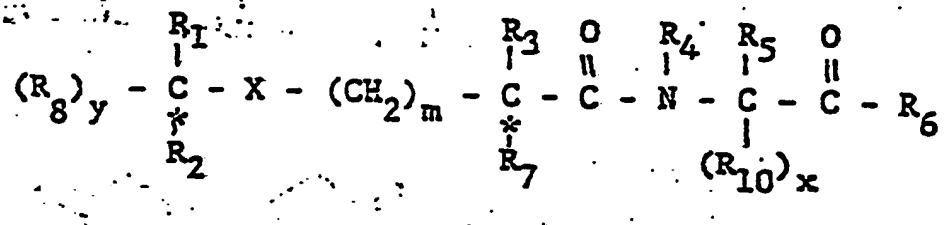
Yours faithfully,

I.B.P.de Minvielle-Devaux.

0073143

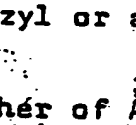
CLAIMS

1. Novel compounds f the general formula:



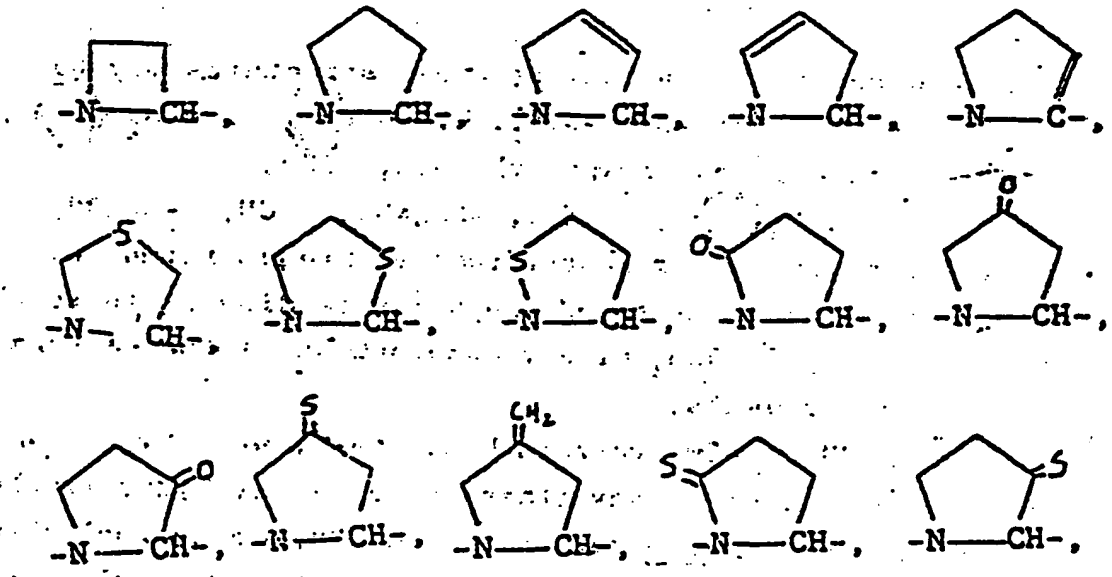
wherein x and y are 0 or 1, X may be S, O or N-R_9 and R_9 may be -H or -CH_3, R_{10} is H, CH_3, F, Cl or Br;

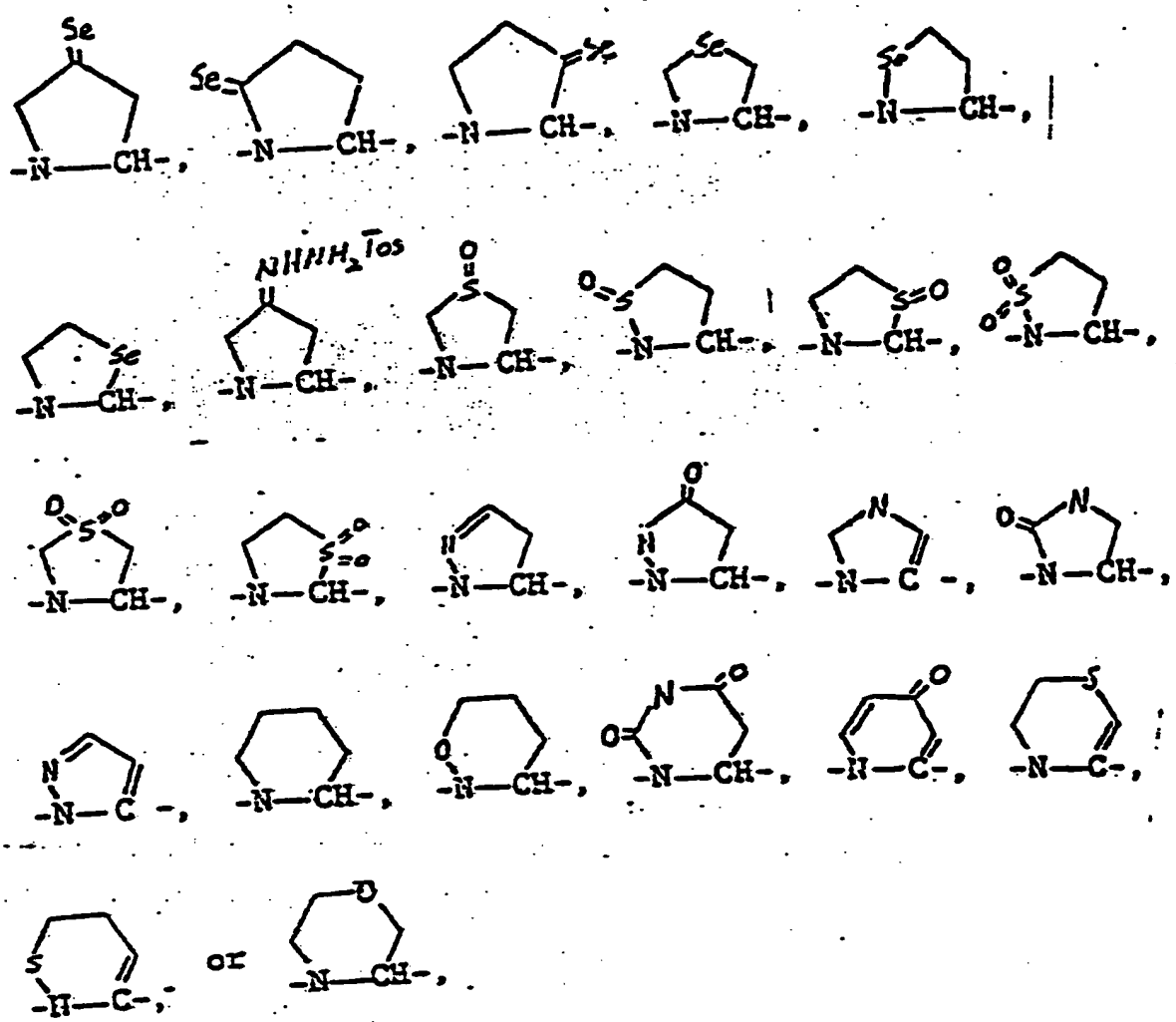
m is 0 or 1;

R_2 is COOH, CH_2COOH, COSH, CH_2COSH, CH_2SH, CH_2CH_2SH, a physiologically acceptable nontoxic salt of any of th m; COOY, CH_2COOY, COSY, CH_2SY, or CH_2CH_2SY wherein Y is phenyl, benzyl or a 1 - 5 carbon alkyl group; or 

wherein either of A_1 and A_2 may be H, phenyl, benzyl or a 1 - 5 carbon alkyl group;

R_4 and R_5 together form a ring with the nitrogen and carbon atoms to which they are respectively attached, which ring is one of the structures:





it being understood that any of these structures may be monosubstituted with -OH, $-OCH_3$, F,  , , Cl, Br, I, phenyl, hydroxyphenyl, -SH, $-SCH_3$, , , $-NHCH_3$, $-CH_2NH_2$, $-CH_3$, $-CH_2OH$, propyl, guanidino, nitro guanidino or thioguanidino and that any of the 5- or 6- membered rings may be disubstituted with -OH, F, Cl, Br, I, OCH_3 or any combination of two of this group of substituents;

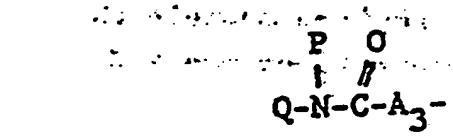
R_6 is -OM or -SM, wherein M may be H, an alkyl group of 1-3 carbon atoms or any other ester moiety hydrolizable under mammalian <u>in vivo</u> conditions to -OH, or an ionically bonded anion of a physiologically acceptable nontoxic salt;

R_7 is H-, CH_3 -, halomethyl, hydroxymethyl, aminomethyl or mercaptomethyl;

R_8 is H-, CH_3-, amino, halomethyl, hydroxymethyl, aminomethyl, dihalomethyl, trihalomethyl, mercaptomethyl, methoxymethyl, methylthiomethyl, methoxycarbonylmethyl, cyanomethyl, benzyl, acetoxymethyl, $CH_2=CH-CH_2$-, isobutyl, mercaptoalkyl of 2-3 carbon atoms, hydroxyalkyl of 2-3 carbon atoms, acetylthioethyl, benzamido, acetamido, phthaloylaminoalkylene wherein the alkylene group has 1-4 carbon atoms, α-alkoxycarbonyl isoalkylene wherein the alkyl group contains 1-5 carbons and the isoalkylene group contains 3 - 5 carbons, benzoylamine, alkanoylamin of 1 - 5 carbons, alkylamide of 1 - 5 carbons, phenylamine, alkylamine of 1 - 5 carbons, or ethyl;

and

A. R_1 and R_3 may each be of the general formula



wherein A_3 is:

I. alkylene of 1-6 carbons, branched chain alkyl of 1-6 carbons, cycloalkylalkylene, alkylcycloalkylalkylen , or alkylcycloalkylene;

II. aralkylene wherein the alkyl group is 1-6 carbons or alkylaryl;

III. phenyl;

IV. alkylaralkylene wherein the alkyl groups may b the same or different and are 1-6 carbons in length;

V. substituted alkylene, substituted branched chain alkyl, substituted cycloalkylalkylene, substituted alkyl cycloalkylalkylene, substituted alkylcycloalkylene, substituted alkylaryl, substituted aralkylene, substitut d phenyl or substituted alkylaralkylene wherein the substitu nt or substituents may be the same or different, may be included in an alkylene chain or pendent thereto, and ar selected from amino, halo, hydroxy, mercapto, NO_2, carboxy, $CONH_2$, lower alkyl, halomethyl, hydroxymethyl, amin methyl, dihalomethyl, trihalomethyl, cyano, mercaptomethyl, methoxy-methyl, methylthiomethyl, methoxycarb nylmethyl, cyan methyl,

benzyl, acetoxymethyl, $CH_2=CH-CH_2-$, i obutyl, mercapto-alkyl of 2-3 carbon atoms, hydr xyalkyl f 2-3 carbon atoms, ac tylthio thyl, b nzamido, c tamido, phthal ylamino-alkylene wherein the alkylene group has 1-4 carbon atoms, α-alkoxycarbonyl isoalkylene wherein the alkyl group contains 1-5 carbons and the isoalkylene group contain 3-5 carbons, benzoylamino, alkanoylamino of 1-5 carbons, alkylamide of 1-5 carbons, phenylamine, alkylamine of 1-5 carbons, lower alkoxy, aryloxy, lower alkylamino, dilow r-alkylamino, acylamino, arylamino, guanidino, imidazolyl, indolyl, lower alkylthio, arylthio, carboxy amido and carbolower alkoxy;

VI. alkylenethio- or alkylenethioalkylene of 1-6 carbons, alkylthioalkylene of 1-6 carbons;

VII. alkyleneoxy or alkyleneoxyalkylene wherein the alkyl groups may be the same or different and are 1-6 carbons;

VIII. alkoxyphenyl or alkoxybenzyl in which the alkoxy group has 1-3 carbons, phenoxyphenyl, phenoxybenzyl, benzyloxybenzyl or benzyloxyphenyl or a thioether analog of any of them;

IX. $-(CH_2)_n-\underset{\underset{OB}{|}}{CH}-(CH_2)_m-$ wherein n=0-4, m=0-4, and B=H or a 1-5 carbon alkyl group; or an -SB analog thereof;

X. $(CH_2)_n-\underset{\underset{O-C-Y}{\|}}{\overset{\overset{O}{\|}}{CH}}-(CH_2)_m$ or $-(CH_2)_n\underset{\underset{C-OY}{\|}}{\overset{\overset{O}{\|}}{CH}}-(CH_2)_m$

or $-(CH_2)_n-\underset{\underset{S-C-Y}{\|}}{\overset{\overset{O}{\|}}{CH}}-(CH_2)_m-$ or $-(CH_2)_n-\underset{\underset{C-SY}{\|}}{\overset{\overset{O}{\|}}{CH}}-(CH_2)_m-$

wherein n and m have the same significance as above, Y is phenyl, benzyl r a 1-5 carbon alkyl group;

XI. $T-\overset{O}{\overset{\|}{C}}-W-$, $T-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-W-$, $-T-\overset{O}{\overset{\|}{C}}-O-W-$,

$-T-\overset{O}{\overset{\|}{C}}-S-W$, $-T-\overset{H}{N}-\overset{O}{\overset{\|}{C}}-W$, $-T-\overset{H}{N}-W-$, $-T-O-\overset{O}{\overset{\|}{C}}-W$,

$-T-S-\overset{O}{\overset{\|}{C}}-W$ wherein T and W may be the same or different and are alkylene; aryl, benzyl or cycloalkyl; and P and Q may be the same, or one of them may be H or th y may combine to form a ring with the nitrogen to which th y are attached.

Either or both of P and Q may be selected from any of the following:

(a) C_1-C_6 straight or branched chain alkyl groups or C_1-C_6 straight or branched chain alkenyl groups, any one of which may be substituted with any of halo, hydroxy, alkoxy, aryloxy, amino, alkylamino, dialkylamino, alkylacylamino, arylamino, guanidino, thioguanidino, nitroguanidino, hydrazino, ureido, nitro, mercaptocarbonyl, hydroxyamino, histidinyl, cyano, imidazolyl, indolyl, mercapto, alkylthi , arylthio, carboxy amido or carboalkoxy, wherein the alkyl groups contain 1-6 carbon atoms;

(b) cycloalkyl or cycloalkylalkylene wherein cycloalkyl has 4-12 carbons, and alkylene 1-5 carbons, which may be substituted with any of -OH, -SH, halo, COOH, COSH, $CONH_2$, NO_2NH_2, NO_2, CH_3, $-OCH_3$, $-\overset{O}{\overset{\|}{C}}-OCH_3$, hydrazine, ureido, $-SCH_3$, hydroxyamino, cyano, guanidino, thioguanidino or nitroguanidino groups;

(c) aralkyl or alkaryl groups which may be ring substituted with one or more of the following:

SH, halo, CH_2COOH, CH_2CONH_2, CH_2CONH-alkyl, COSH, COOH, $CONH_2$, CONH-alkyl, CH_2COSH, CH_2SH, CH_2OH, OH, NO_2, amino, alkyl, alkoxy, aralkyloxy, alkylthio, and aralkylthio groups, wherein the alkyl groups contain 1-6 carbons and may also alternatively be chain substituted with $-CH_3$, -OH, $-OCH_3$,

Hal, $-SCH_3$, $-\overset{O}{\overset{\|}{C}}-CH_3$, $-NH_2$, NO_2, -CN, -SH, $-NHNH_2$, $NH-\overset{O}{\overset{\|}{C}}-NH_2$, -NHOH or a thi or nitro derivative th reof, -COOH, or COSH;

(d) an aryl, heterocyclic or adamantanyl group which may be ring-substituted with at least one group selected from halo, -OH, -O-alkyl, -O-aryl, NH_2, NH-alkyl, $N-(alkyl)_2$, $alkyl-\overset{O}{\overset{\|}{C}}-NH_2$, $aryl-NH_2$, guanidino, thioguanidino, nitroguanidino, hydrazino, ureido, nitro, mercaptocarbonyl, hydroxyamino, cyano imidazolyl, indanyl, histidinyl, -SH, -S-alkyl, S-aryl, $-\overset{O}{\overset{\|}{C}}-NH_2$, $-\overset{O}{\overset{\|}{C}}-O-alkyl$, $-\overset{O}{\overset{\|}{C}}-alkyl$, $-\overset{O}{\overset{\|}{C}}-O-aryl$, $-\overset{O}{\overset{\|}{C}}-aryl$, $-\overset{O}{\overset{\|}{C}}-SH$, $-\overset{O}{\overset{\|}{C}}-S-alkyl$, $-\overset{O}{\overset{\|}{C}}-S-aryl$ and $-NO_2$,

when P and Q join with N to form a ring, the ring may be any 4 - 10 membered heterocyclic ring which contains a nitrog n with only two of its valences attached to other ring members.

B. Alternatively R_1 may be

$$Q-\overset{P}{\overset{|}{N}}-\overset{O}{\overset{\|}{C}}-A_3-$$ and

R_3 may be

(i) mono-N substituted alkylene of 2 - 4 carbons wherein the N substituent is benzoyl, Boc, CbO, Tos, formyl or acetyl;

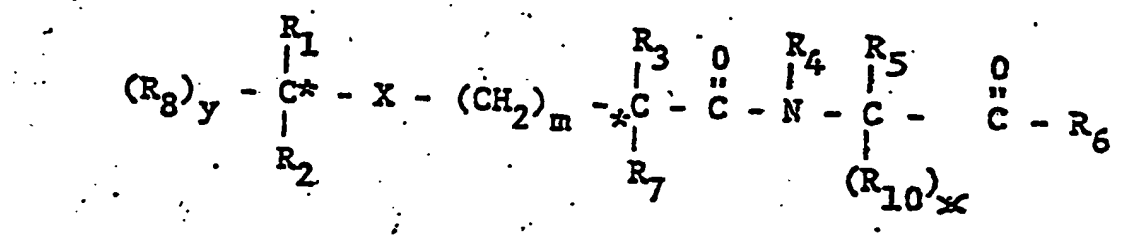
(ii) hydroxyphenyl or hydroxyphenyl-(1-6C)-alkylene r a thiol analog of either;

(iii) mercaptoalkylene of 1 - 6 carbons;

(iv) phenylalkylene wherein the alkylene group has 1-6 carbons;

(v) phenylthioalkylene or benzylthioalkylene wherein the alkylene group has 1 - 6 carbons;

(vi) alkylthioalkylen wherein th alkyl and alkylene groups have 1 - 3 carbons;

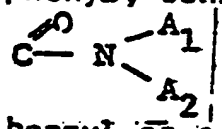


Wherein x and y are 0 or 1, X may be S, O or N-R_q and R_q may be -H or $-CH_3$;

R_{10} is H, CH_3, F, Cl or Br;

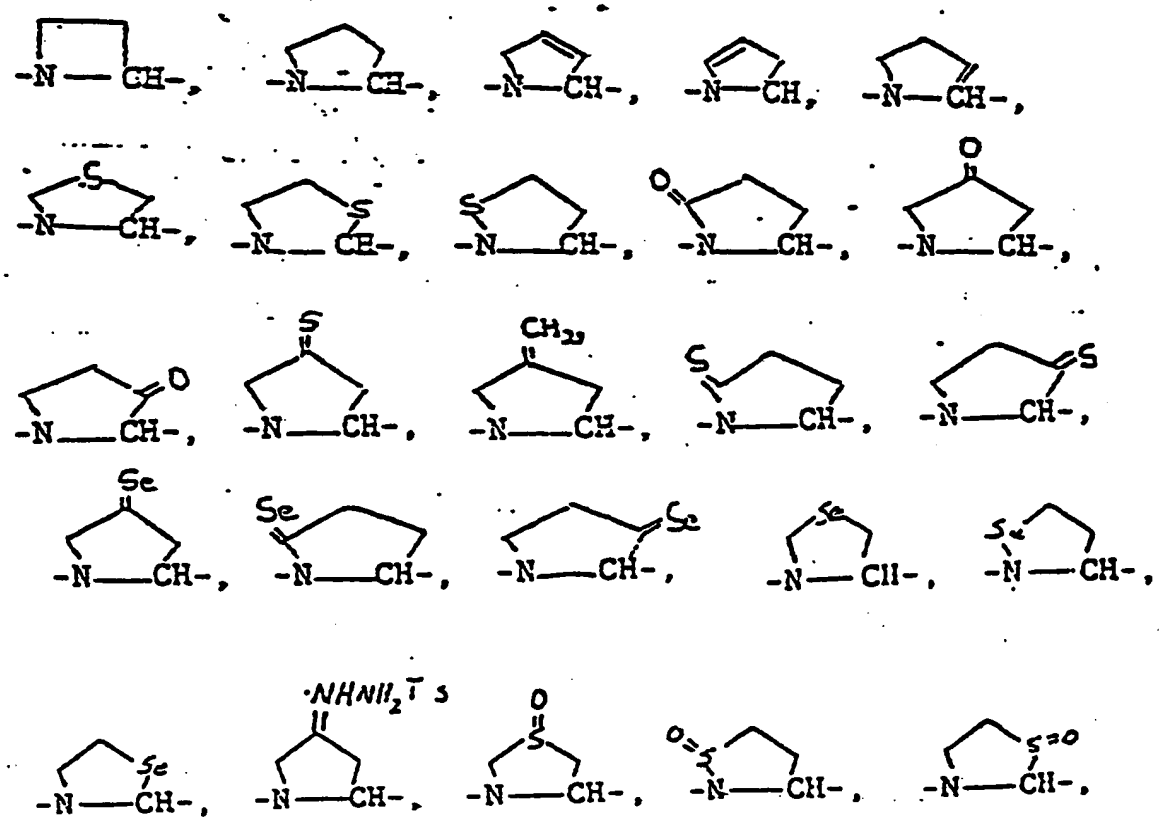
m is 0 or 1,

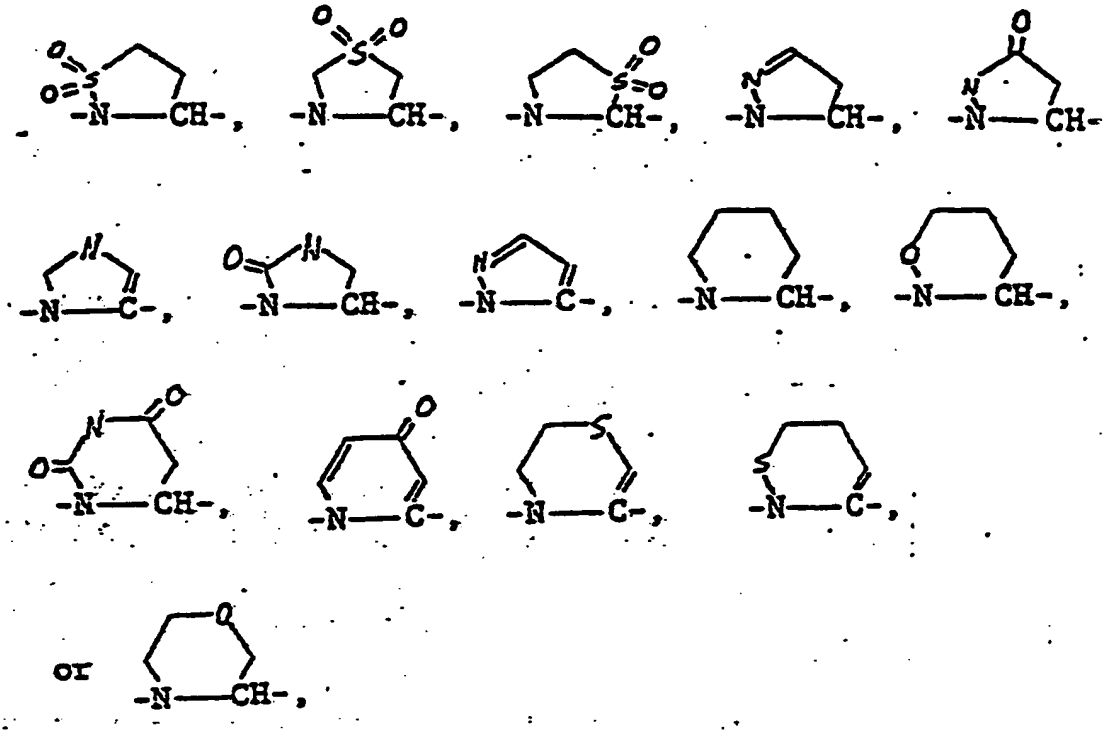
R_2 is COOH, CH_2 COOH, COSH, CH_2COSH, CH_2SH, CH_2, CH_2SH, a physiologically acceptable nontoxic salt of any of them; COOY, CH_2 COOY, COSY, CH_2SY, or CH_2CH_2SY wherein Y is phenyl, benzyl or a 1-5 carbon alkyl group, or



wherein either of A_1 and A_2 may be H, phenyl, benzyl or a 1-5 carbon alkyl group;

R_4 and R_5 together form a ring with the nitrogen and carbon atoms to which they are respectively attached, which ring is one of the structures:





or

$G-(CH_2)_q-\overset{OH}{\overset{|}{C}}H-(CH_2)_n-$, $NH_2-\overset{O}{\overset{||}{C}}-(CH_2)_q-\overset{O}{\overset{||}{C}}-(CH_2)_n-$,

$r-NH_2-\overset{O}{\overset{||}{C}}-(CH_2)_q\overset{OH}{\overset{|}{C}}H-(CH_2)_n-$,

wherein G is an alkacyl or alkacyloxy group of 1 - 6 carbons, a benzoyl or benzoyloxy group, or a phenylalkacyl or phenylalkacyloxy group wherein the alkacyl or alkacyloxy group contains 2 - 6 carbons and q and n have the same significanc as set forth above;

(xx) $K-(CH_2)_n-\overset{O}{\overset{||}{C}}-(CH_2)_n-$ or $K-(CH_2)_n-\overset{OH}{\overset{|}{C}}H-(CH_2)_n-$ wherein n has the significance stated above and K is selected from carboxyphenyl, aminophenyl, nitrophenyl, halophenyl, hydroxyphenyl, alkylthiophenyl, alkylphenyl, mercaptophenyl, cyanophenyl, mercapto-carbonylphenyl, alkylcarbonylphenyl, alkylcarbonyloxyphenyl, hydrazinophenyl, ureidophenyl, alkylcarbonylaminophenyl, alkylcarbonylthiophenyl, alkyloxyphenyl and hydroxyaminophenyl, wherein all alkyl groups contain 1 - 6 carbon atoms;

(xxi) $L-(CH_2)_n-\overset{O}{\overset{||}{C}}-(CH_2)_n-$ or $L-(CH_2)_n-\overset{OH}{\overset{|}{C}}H-(CH_2)_n-$ wherein n has the significance stated above and L is selected from cycloalkyl groups of 3 - 7 carbons which may b unsubstituted or substituted with up to two groups selected from among carboxy, amino, nitro, halo, hydroxy, mercapto, mercaptocarbonyl, hydroxyamino, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkylthio, alkylcarbonylamino, alkylcarbonylthio, cyanohydrazino, ureido and alkyloxy, wherein all alkyl groups contain 1 - 6 carbon atoms:

(xxii) guanidino alkylene, thioguanidinoalkylene, or nitroguanidino alkylene in which the alkylene groups contain 1 - 6 carbon atoms;

(xxiii) ring substituted aryl groups in which the ring substituents may be the same or different and may comprise up to five per ring of the following: $-NH_2$, -OZ, -SZ, halogen, -CN, $-NO_2$, -COOZ, COSZ, $CONH_2$, $-NHNH_2$, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkylcarbonylamino,

haloalkyl, dihaloalkyl, trihalomethyl, hydroxyamino, alkylcarbonylthio, phenoxy, and benzyloxy wherein the alkyl groups contain 1 - 6 carbon atoms and Z has the same significance as above;

(xxiv) amidoalkylene or alkylcarbonyl-aminoalkylen wherein the alkyl and alkylene groups contain 1 - 6 carbon atoms;

(xxv) hydroxyaminoalkylene of 1 - 6 carbons;

(xxvi) vinyl and substituted vinyl groups in which the substituents may be alkyl, aryl, cycloalkyl or heterocyclic groups;

(xxvii) unsubstituted heterocyclic groups from among phenothiazinyl, pyrrolidinyl, pyrrolyl, quinolinyl, imidazolyl, pyridyl, thyminyl, benzothiazinyl, indolyl, thienyl, purinyl, piperidinyl, morpholinyl, azaindolyl, pyrazinyl, pyrimidyl, piperonyl, piperazinyl, furanyl, thiazolyl and thiazolidinyl, cytosinyl;

(xxviii) alkylene or alkenyl groups 1 - 6 carbons substituted with one of the heterocyclic rings from (xxvii) above;

(xxix) groups from (xxvii) or (xxviii) above containing up to four ring substituents on the heterocyclic ring selected from among - OZ, - SZ, - COOZ, - NO_2, - NH_2, - COSZ, halogen, haloalkyl, dihaloalkyl, trihalomethyl, cyano, $CONH_2$, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkylcarbonylthio, phenoxy, benzyloxy, -NH - $\overset{O}{\overset{\|}{C}}$ - NH_2, - $NHNH_2$ and HONH -, wherein Z has the same significance as above;

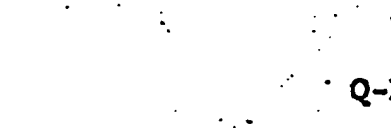
(xxx) groups from (xxvii), (xxviii) or (xxix) attached to one valence of an etheric -O- or -S-;

(xxxi) mono-, di- or tri-alkyl, alkenyl - or phenylsilyl or -selenyl wherein the alkyl or alkenyl groups contain 1 - 6 carbons;

(xxxii) any of H, 1 - 5 carbon straight or branched chain alkyl, phenyl, -OH, alkoxy of 1 - 6 carbons, benzyloxy, benzyloxyalkylene or phenoxyalkylene wherein the alkylen has 1 - 5 carbons, alkoxyalkylene having 1 - 5 carbons in the alkoxy and alkelene groups, aminoalkylene of 1 - 6

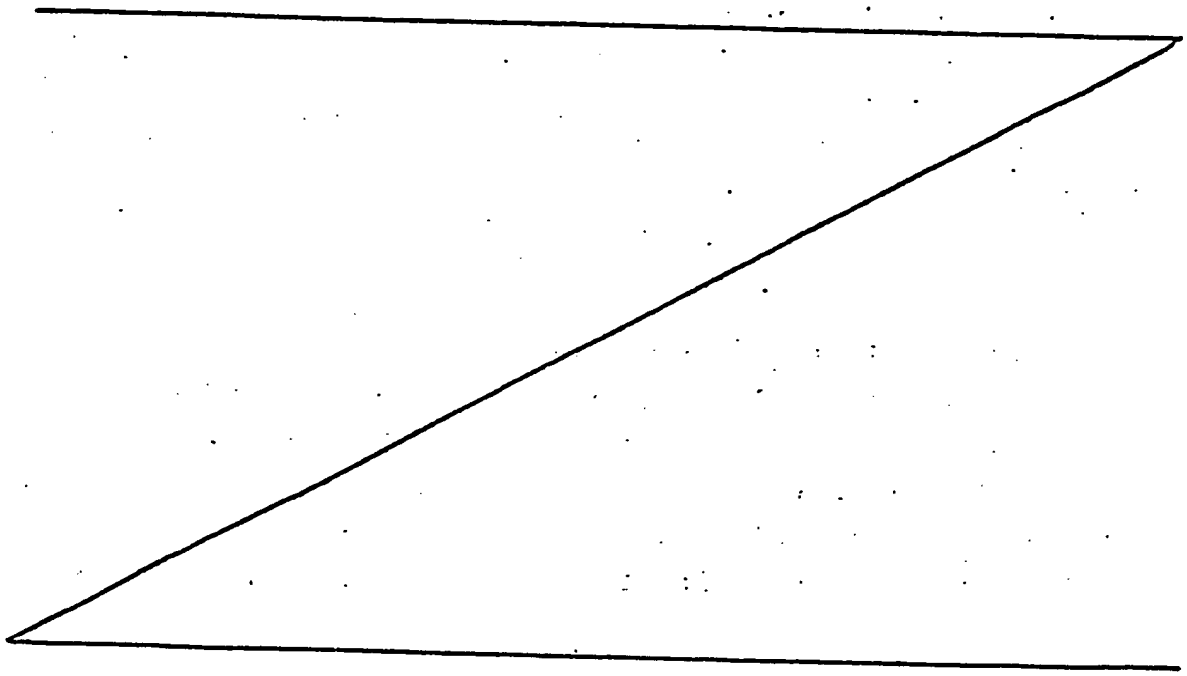
carbons, alkenyl of 1 - 6 carbons, benzyl, hydroxyalkyl of 1 - 6 carbons, mercaptoalkyl of 1 - 6 carbons, histidinyl, haloalkyl of 1 - 6 carbons, 4 - aminomethyl-benzyl, acetamidoalkyl of 1 - 5 carbons, benzylthiomethylene, or dimethylaminoalkyl of 1 - 5 carbons.

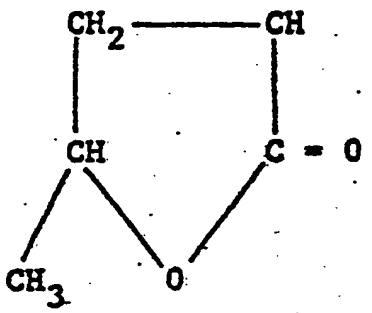
C. Alternatively, R_3 may be



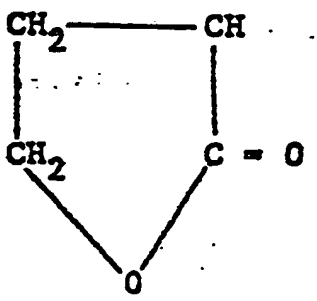
and

R_1 may be any of groups (i) - (xxxii) above or any of H, C_1 - C_8 straight or branched chain alkyl, phenyl, benzyl, unsubstituted aminoalkylene of 2 - 6 carbons, hydroxyalkylene of 1 - 6 carbons, hydroxyphenyl, phenoxyalkylene or benzyloxyalkylene wherein the alkylene group has 1 - 6 carbons, cycloalkyl of 3 - 6 carbons, cycloalkyl methyl, 3 indolyl, phenylethyl, methylthioethyl, 3 indolyl alkyl wherein the alkyl group contains 1 - 5 carbons, imidazolyl, imidazolylalkyl wherein the alkyl group contains 1 - 5 carbons, phenoxymethyl, phenylthiomethyl, 4-aminomethyl benzyl, 2-aminophenethyl, naphthylethyl, 4-halophenethyl, 3, 4-dihalophenethyl or phenoxyphenethyl, or R_1 and R_2 together may form with -CH a lactone ring of the formula:





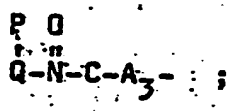
or



or an analogous six-membered ring.

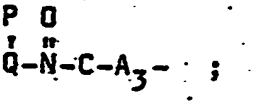
2. A compound according to claim 1 wherein X is -NH-, -S- or -O-.

3. A compound according to claim 1 or 2 wherein R_1 and R_3 are each of the general formula



and P and Q may be the same, or one of them may be H or they may combine to form a ring with the nitrogen to which th y are attached, wherein P and Q may be selected from any of the radicals of the groups (a) - (d).

4. A compound according to claim 1 or 2 wherein R_1 is of the general formula



R_3 is a radical of groups (i) - (xxxii);

and P and Q may be the same, or one of them may be H of they may combine to form a ring with the nitrogen to which they are attached, wherein P and Q may be selected from any of the radicals of groups (a) - (d).

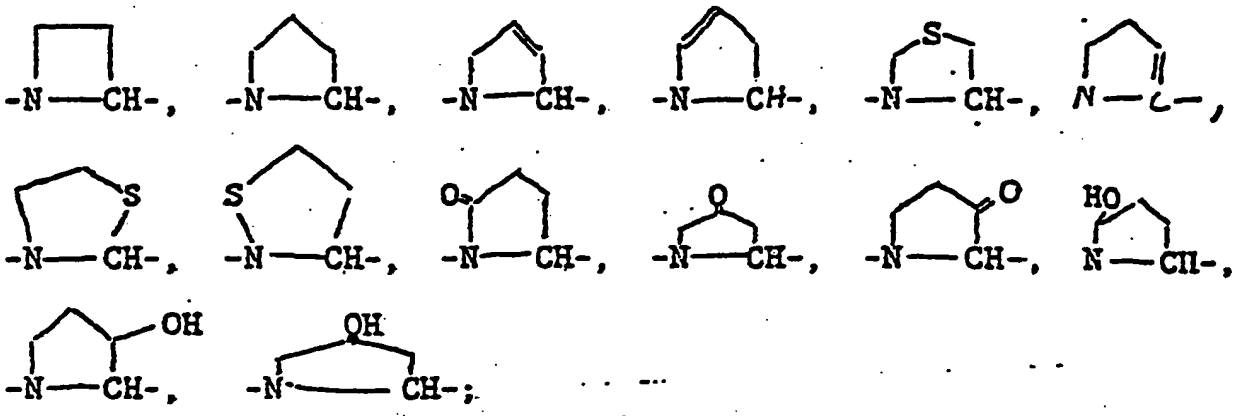
5. A compound according to any of claims 1 t 4 wherein

m is 0;

X is N-R_9 and R_9 is H;

R_4 and R_5 togeth r form a ring with the nitrogen and

carb n at ms to which they are r spectively attach d, which ring i one of th tructures:

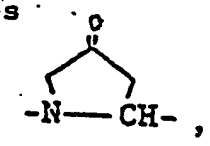


R_7 is H- or CH_3-;
R_8 is H- or CH_3-; and
R_{10} is H- or CH_3-.

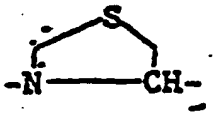
6. A compound according to claim 5 wherein
A_3 is a radical of groups I - V; and
P and Q are the same or different and are selected from H and any radical of groups (a) - (d).

7. A compound according to claim 5 wherein
A_3 is a radical of groups I - V;
P and Q are the same or different and are selected from radicals of the groups (b) - (d),
when P and Q join to form a ring, the ring is any 4 - 10 membered heterocyclic ring which contains a nitrogen with only two of its valences attached to other ring numbers.

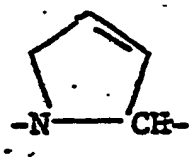
8. A compound according to any of claims 1 - 7 wherein R_4 and R_5 form one of the structures







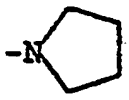




(where h is Cl, F, Br or I), and R_6 is -OH, or a lower alkyl ester or physiologically acceptable salt thereof.

9. A compound according to any of claims 1 - 7 wher in R_4 and R_5 and R_6 together form substituted proline, or a lower alkyl ester thereof and physiologically acceptable salts ther - of, wherein the substituent is selected from the group consist ing of Cl, Br, F or I.

10. A compound according to any of claims 1 - 9 wherein P-N-Q form the structure



and x = 1, y = 1, X = N-R_9, m = 0, R_8 = H, R_3 = CH_3, R_4 and R_5 together form the structure

R_6 = OH, R_7 = H and R_2 = COOH, R_9 = H, R_{10} = H or physiologically acceptable salts thereof.

11. A compound according to any of claims 1 - 9 wherein P = H, Q = phenyl or iodo-phenyl, x = 1, y = 1, X = NR_9, m = 0. R_8 = H, R_3 = CH_3, R_4 and R_5 together form the structure

R_6 = OH, R_7 = H and R_2 = COOH, R_9 = H, R_{10} = H or physiologically acceptable salts thereof.

12. A compound according to claim 1 wherein R_2 is COOH, COOEt, COOMe, $CONH_2$, COSH, CH_2SH; or wherein P-N-Q forms structures selected from the group consisting of anilino, benzylamino, 2-amino pyridyl amino, 3-amino pyridyl amino, 4-amino pyridyl amino, 3-indolyl amino, and histamino; or wherein R_3 is CH_3.

13. A compound according to any of claims 1 - 12 wherein

P of R_1 is H;

Q of R_1 is aminoalkylene;

A_3 of R_1 is alkylene; and

P-N-Q of R_3 forms structures selected from the group consisting of anilino, benzylamino, 2-amino pyridyl amino, 3-amino pyridyl amino, 4-aminopyridyl amino, 3-indolyl amino, and histamino; or wherein

P of R_3 is H,

Q of R_3 is aminoalkylene;

A_3 of R_3 is alkylene;

P-N-Q of R_1 forms structures selected from the group consisting of anilino, benzylamino, 2-amino pyridyl amino, 3-amino pyridyl amino, 4-amino pyridyl amino, 3-indolyl amino, and histamino; or wherein

R_1 is $Q\text{-}N(P)\text{-}C(=O)\text{-}A_3$

R_3 is phenyloxyalkylene, benzyloxyalkylene, benzylalkyleneoxyalkylene, wherein the alkylene group has 1 - 5 carbons; or wherein

R_1 is phenyloxyalkylene, benzyloxyalkylene, benzylalkylene oxyalkylene, wherein the alkylene group has 1 - 5 carbons;

R_3 is $Q\text{-}N(P)\text{-}C(=O)\text{-}A_3$.

14. A composition of matter effective to inhibit angiotensin converting enzyme in vivo or to reduce the blood pre sure in vivo of a mammal in a hypertensive state which contains as its essential activ ingredient a therapeutically effective amount of a compound of any of claims 1 - 13.

15. A compound of any f claims 1 - 13 for us in tr ating hypertension or abn rmal s rum levels of angiot nsin II in mammals.